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ANXIOLYTIC-LIKE EFFECT OF 2-(4-((1-PHENYL-1H-PYRAZOL-4-YL)METHYL)PIPERAZIN-1-YL)ETHAN-1-OL IS MEDIATED THROUGH THE BENZODIAZEPINE AND NICOTINIC PATHWAYS

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ABSTRACT

In this study we proposed the design, synthesis of a new compound 2-(4-((1-phenyl-1H-pyrazol-4-yl)methyl)piperazin-1-yl)ethan-1-ol (LQFM032), and pharmacological evaluation of its anxiolytic-like effect. This new compound was subjected to pharmacological screening refered to as Irwin test, prior to sodium pentobarbital-induced sleep, open field and wire tests. The anxiolytic-like effect of this compound was evaluated using elevated plus maze and light-

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dark box tests. In addition, the mnemonic activity was evaluated through step-down test. In sodium pentobarbital-induced sleep test, LQFM032 decreased latency and increased duration of sleep. In the open field test, LQFM032 altered behavioral parameter, that sugested anxiolytic-like activity, as increased in crossings and time spente at the center of open field. In the plus maze-test and light-dark box test, the LQFM032 showed anxiolytic-like activity, increased entries and time spent on open arms, and increased in number of transitions and time spent on light area, respectively. Those effects was antagonized by flumazenil but not with 1-(2-Methoxyphenyl)-4-(4-phthalimidobutyl)piperazine (NAN-190). The LQFM032 did not alter mnemonic activity. Moreover, the anxiolytic-like activity of LQFM032 was antagonized by mecamilamine. In summary, LQFM032 showed benzodiazepine and nicotinic pathways mediated anxiolytic-like activity without altering the mnemonic activity.

Keywords: benzodiazepine site, nicotinic activity, piperazine derivative, LQFM032.

1.0. INTRODUCTION

Diseases that affect mental health are common, for example, in the US in 2014 it was estimated that 43.6 million adults in US (18.1% of Americans) had mental disorder [1, 2, 3, 4, 5]. Mental disorders are associated with social-economical problems and high annual medical cost that exceeds US $ 317 billion [3, 4].

Mental disorders are generally characterized by changes in mood, thinking and/or behavior. Nowadays, anxiety is among the most leading mental disorders [5]. Although anxiety could be beneficial (in a physiological situation), excessive anxiety could lead to suffering and affect daily activities. Symptoms such as difficulty concentrating, irritability, muscle tension and trouble sleeping are commonly found among anxiety patients [1, 6].

Currently, anxiety is being treated with different classes of anxiolytics drugs. Benzodiazepine such as diazepam and clonazepam are effective agents but their clinical application is associated with tolerance, the risk of abuse, anterograde amnesia [7, 8] among other undesirable effects. For this reasons, the benzodiazepines are not considered as the first choice of treatment[1, 9]. The selective serotonin reuptake inhibitors, and dual selective inhibitors of the reuptake of serotonin and norepinephrine, seems to be prefered as the first line of therapy[9]. However, it takes weeks of consistent administration of these drugs to achieve anxiolytic effects. Hence, further research with the aime of developing effective treatments with fewer side effects becomes a necessity[10].

In the current study, we describe the biological evaluation of a new heterocyclic piperazine derivative, LQFM 032 (**4**), which was designed from hybridization molecular strategy from LQFM008 (**2**) and JWB-1-84-1 (**3**) - lead compounds. LQFM 008 (**2**) is an anxiolytic and antidepressive-like compound[11, 12] that was synthesized through molecular simplification of clozapine (**1**), while JWB-1-84-1 (**3**) is an analogue of acetylcholine with neuroprotective properties [13]. As illustrated in **Figure 1A**, we changed ethyl 1-peperazinacarboxylate C by 1-(2-hydroxyethyl)piperazine C.

2. Material and Methods

2.1. Chemical synthesis

2.1.1. General methods

Reactions were monitored by TLC using commercially available precoated plates (Whatman 60 F254 silica) and developed plates were examined under UV light (254 and 365 nm). ^{1}H and ^{13}C NMR spectra were recorded in the indicated solvent on Bruker Avance III 500 MHz spectrometer. Chemical shifts were quoted in parts per million downfield from TMS and the coupling constants in Hertz. Infrared spectra were recorded on a Perkin- Elmer Spectrum Bx-II FT-IR System spectrophotometer instrument as films on KBr discs. Melting points were performed using a Marte melting point apparatus, and the results were uncorrected. All assignments of the signals of ^{1}H and ^{13}C NMR spectra are consistent with the chemical structures of the products described. The organic solutions were dried over anhydrous sodium sulfate and organic solvents were removed under reduced pressure in a rotary evaporator. Mass spectra (MS) were obtained with a QQ-TOF (Brucker Daltonics Bremen, Germany). The sample preparation for MS analysis consisted of diluting 1 mg of each sample in 1 mL of methanol. In order to perform the analysis in positive and negative mode, 1 μL of formic acid and 1μL of ammonium hydroxide, respectively, were added to the samples. The solution obtained was directly infused at a flow rate of 2μL/min into the ESI source. The ESI (±) source conditions were as follows: a nebulizer gas pressure of 0.5 - 1.0 bar, a capillary voltage of 3.0 kV and a transfer capillary temperature of 250 °C.

2.1.2. Synthesis of 2-(4-((1-phenyl-1*H*-pyrazol-4-yl)methyl)piperazin-1-yl)ethanol (4)

5 mL of MeOH was added in ZnCl2 (0.5 mmol). After was added this compounds in aldehyde (**6**) (1.0 mmol), that contained 1-(2-hydroxyethyl)piperazine **(7)** (1.0 mmol) and $NaBH_3CN$ (0.5 mmol) **(Figure 1B)**. The mixture was stirred at reflux temperature for 2 hours[14]. In tu rn, MeOH was then evaporated and the residue was partitioned between water

and CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), concentrated in vacuum, and the crude product was purified through chromatography method with hexane:ethyl acetate as mobile phase to provide 2-(4-((1-phenyl-1*H*-pyrazol-4-yl)methyl)piperazin-1-yl)ethanol (**4**) as a beige oil in 73.0% of yield, *Rf*=0.38 (dichloromethane/methanol, 90:10): IR_{max} (KBr) cm^{-1}: 3392 (*v* O-H), 1559 (*v* C=C aromatic) and 1402 (*v* CH_2) **(Figure 2A)**; ^{1}H-NMR (500.13 MHz **Figure 2B)** $CDCl_3$ δ: 7.90 (1H, s, H-5), 7.67 (2H, m, H-2' and H-6'), 7.64 (1H, s, H-3), 7.44 (2H, m, H-3' and H-5'), 7.27 (1H, m, H-4'), 3.66 (2H, t *J*=5.28, H-8''), 3.54 (2H, s, H-6), 2.98 (1H, s, H-9''), 2.67 (8H, m, H-2'', H-3'', H-5'' and H-6''), 2.62 (2H, t, *J*=5.28, H-7''); ^{13}C-NMR (125.76 MHz – **Figure 2C**) $CDCl_3$ δ: 141.8 (C-3), 140.0 (C-1'), 129.4 (C-3' and C-5'), 126.7 (C-4'), 126.4 (C-5), 118.9 (C-2' and C-6'), 118.8 (C-4), 59.4 (C-7''), 57.6 (C-8''), 52.8 (C-2'', C-3'', C-5'' and C-6''), 52.3 (C-6). MS: $[M+ H]^+$ *m/z* of 287.189 **(Figure 2D)**.

2.2. Pharmacological evaluation

2.2.1. Drugs and treatments

The control group received polisobarte 80 2% (vehicle – Tween 80 – Synth, USA) at the dose of 10 mL/Kg. LQFM032 (2-(4-((1-phenyl-1H-pyrazol-4-yl)methyl)piperazin-1-yl)ethan-1-ol – Molecular Weight - MW: 286.18 mg/mmol) was administered by routes p.o., s.c. and i.p., at the doses between 7.0 - 875 µmol/Kg for Irwin test. In specific tests, like open field and plus maze, LQFM032 was administered orally at doses of 18, 54 and 162 µmol/Kg. LQFM032 was dissolved in Tween 80 2%. Sodium pentobarbital (MW: 226.26 mg/mmol, Sigma Chemical Co., USA) was administred at the dose of 220 µmol/Kg. Flumazenil (MW: 303.29 mmol/mg, União Química, Brazil), benzodiazepine site competitive antagonist, was administred at the dose of 6.6 µmol/Kg i.p., 1-(2-methoxyphenyl)-4-[4-(2-phthalimido) buthyl] piperazine hydromide (NAN-190 – MW: 393.48 mg/mmol, Chemical Co., USA), 5-HT_{1A} competitive antagonist, was administred at the dose of 1.3 µmol/Kg i.p. To verify the pharmacological effects of benzodiazepine site and 5-HT_{1A} receptor agonists, diazepam (MW: 284.73 mg/mmol, Cristália, Brazil) at a dose of 3.51 µmol/Kg p.o. and buspirone (MW: 385.50 mg/mmol, Sigma, USA) at a dose of 26 µmol/Kg p.o. Scopolamine (MW: 303.34 mmg/mmol, Sigma, USA), muscarinic receptor antagonist was administered at dose of 1.0 µmol/Kg i.p. to induce memory.

2.2.2. Animals

Adult male Swiss mice weighing approximately 30 g, six weeks old, were used in all experiments. These animals were provided by the Central Animal House of Federal University of Goiás (UFG). All experimental groups, except for Irwin test, had eight mice per group. The animals were housed in groups of 20 mice/cage (50 x 23 x 16 cm) and were maintained under controlled conditions of temperature (25±2ºC) with a 12 h dark/light cycle, with food and water *ad libitium*. The tests were conducted between 8:00 a.m. and 4:00 p.m. All experiments were carried out according to the Ethical Principles in Animal Research as adopted by the Brazilian Society of Laboratory Animal Science and approved by the Ethics Committee on Animal Use of University Federal of Goiás-Brasil (nº 023/13).

2.2.3. Effects on gross behavior or Irwin test

Experimental groups of mice (n=3 per group) were treated orally (p.o.), intraperitoneally (i.p.) or subcutaneously (s.c.) with LQFM032 at doses of 7, 35, 175 and 875 µmol/Kg whereas control group received vehicle 10 mL/Kg by the same routes. The animals were observed in free ambulation on a flat surface for 3 min, at 0, 5, 10, 20, 30 and 60 min, 4, 8, 24 and 48 h, 4 and 7 days after the treatment. The observed effects were noted using a standard pharmacological screening approach [15].

2.2.4. Sodium pentobarbital-induced sleep test

Experimental groups of 8 mice each were treated orally with vehicle 10 mL/Kg or LQFM032 at doses of 18, 54 and 162 µmol/Kg. Sixty minutes after the treatment, the animals received sodium pentobarbital (220 µmol/Kg, i.p.). Latency to induce sleep (loss of the rigthing reflex) and the duration of the sleep (the time required to recover the righting reflex) were recorded for each animal [16].

2.2.5. Open field test

The apparatus consisted of a circular arena measuring 36 cm (diameter) x 20 cm (height), with the floor divided into eight squares of equal area. Mice (n=8) were individually placed at the center of the open field apparatus to measure during 5 min locomotor activity after 60 min of oral treatment with vehicle or LQFM032 (18, 54 and 162 µmol/Kg). The number of squares crossed, rearing behaviors, immobility time (s), the number of crossings and time (s) spent at the center (%CrCe and TCE) of the arena were recorded [17, 18].

2.2.5. Wire hanging test

Groups of mice (n=8) were treated orally with vehicle or LQFM032 (18, 54 and 162 µmol/Kg). One hour after treatment, animals were suspended from an elevated wire by their forepaws at a height of ~20 cm above the floor to prevent the animal from climbing down. The animal was placed at the center of the wire. The time that elapsed until the animal fell was recorded three times and the cutoff time was set at 60 s. The latency to the falls was recorded and analyzed [16].

2.2.6. Elevated plus maze test (EPM)

The EPM apparatus consisted of two open arms (30 cm x 5 cm x 0.5 cm) and two closed arms (30 cm x 5 cm x 15 cm) connected by a commom central platform (5 cm x 5 cm). The experimental groups of 8 mice each that were treated p.o. with vehicle or LQFM032 (18, 54 and 162 µmol/Kg) were exposed to EPM apparatus. Sixty minutes after the treatment, mice were placed individually at the center of the EPM facing one of the enclosed arms and observed for 5 min [19, 20]. The test was carried out under a red light (15 W) and was fully recorded for later analysis. Parameters such as number of entries (EOA) and the time spent in the open arms (TOA) of EPM were used as a measure of anxiety. Anxiolytic compounds reduce the animal's aversion to the open arms and promote its exploration.

2.2.7. Light-dark box test (LDB)

Experimental groups (n=8) mice were treated p.o. with vehicle or LQFM032 (18, 54 and 162 µmol/Kg). Sixty minutes after the treatment animals were placed at the center of the light area (20 x 26 x 26 cm) facing the opening (7 cm x 7 cm) of the dark area (20 cm x 26 cm x 17 cm). The number of transitions between the compartiments and the time spent in the light area (TLA) were recorded over a 5 min [21].

2.2.8. Mechanism of anxiolytic like property

In order to investigate the possible mechanisms underlying the anxiolytic-like activities of LQFM032, the animals were pre-treated i.p., with saline 0.9% (10 mL/Kg), flumazenil (6.6 µmol/Kg) or NAN-190 (1.3 µmol/Kg). After 30 min, the animals were treated p.o. with vehicle, LQFM032 (54 µmol/Kg), diazepam (3.51 µmol/Kg) or buspirone (26 µmol/Kg). Sixty minutes after the last treatment, mice were submited to the light-dark box test.

2.2.9. Effects of LQFM032 on avoidance memory in stepdown test

The stepdown avoidance test was used to evaluate the possible effect of LQFM032 on cognition. The animals were submitted to one training session, after 60 min of treatment p.o. with vehicle or LQFM032 (18, 54 or 158 µmol/Kg, p.o.). The mice were placed on the platform and the stepdown from the platform with all four paws was immediately followed by a foot-shock (0.5 mA) for 1 s. The latency to stepdown was recorded. Afteward, the animals were exposed to the apparatus again (test session) after an interval of 90 min and 24 h from the first exposure, training session (TS). The test session was conducted in the absence of shock and the stepdown latency from the platform was recorded (up to 180 s) and evaluated as indicative of memory retention [22].

In order to evaluate the capacity of LQFM032 to prevent scopolamine-induced memory deficits, the animals were pre-treated p.o., 30 min before, with LQFM032 (18 µmol/Kg) or vehicle. Then, animals were treated i.p. with saline or scopolamine 1.0 µmol/Kg. Thirty minutes after the last treatment, the animals were exposed to TS, and the the latency to step-down (s) was recorded.

2.2.10 Evaluation of nicotinic receptor participation in anxiolytic-like activity of LQFM032

In order to verify the involvement of nicotinic receptor in the anxiolytic-like activity of LQFM032, the animals were pre-treated i.p. with saline or mecamylamine 30 µmol/Kg. Then, 30 min after, were administred p.o. vehicle or LQFM032 54 µmol/Kg. One hour after the last treatment, LDB test was realized and the time spent in the light area and number of transitions between compartments were recorded.

2.2.11 Statistical analysis

Results were expressed as means±standard error of mean (SEM). Data was analyzed by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls as the post-hoc test. In the wire hanging test, the data were analyzed by the Kruskal-Wallis test followed by Dunns as *post hoc* test and expressed as median (25^{th} percentile to 75^{th} percentile). Effects were considered significant at $p \leq 0.05$. All graph was drawn using GraphPad Prism version 5.0® software.

3.0. Results

3.1. Synthesis of LQFM032

As illustrated in **Figure 1B**, the synthetic route began with 1-(phenyl)-1*H*-pyrazole (**5**) and proceeded through the classical method described by Finar and Godfrey, in 88% of yield (Finar, 1957). Chemoselective and regiospecific formylation of 1-(phenyl)-1*H*-pyrazole (**5**) to 1-(phenyl)-1*H*pyrazole-4-carbaldehyde (**6**) was performed under Duff's conditions in 83% of yield (de Oliveira, 2013). The synthesis of 2-(4-((1-phenyl-1*H*-pyrazol-4-yl) methyl) piperazin-1-yl) ethanol (**4**) was carried out under conditions of reductive amination in 73 % of yield (Lou, 2004). LQFM032 (**4**) was obtained in three synthetic steps with 53 % overall yield.

3.2. Effects on gross behavior or Irwin test

Animals treated with LQFM032 at the dose of 7 µmol/Kg (p.o., i.p. or s.c.) did not altered behavioral activity. However, LQFM032 at dose of 35 µmol/Kg (s.c.) decreased exploratory activity after 5 minutes of administration. Treatment at dose of 175 µmol/Kg (s.c.) induced ataxia and lethargy. The same dose through i.p. or p.o. increased exploratory activity. Finally, at dose of 875 µmol/Kg (p.o., i.p. or s.c.) behavioral parameters such as sedation, diarrhea, paralysis of the hindlimbs were observed. All behavioral alterations (induced by LQFM032 treatment at diferents doses and routes) ceased after 4 days without any death record at the end of the 7 days of observation.

3.3. Sodium pentobarbital-induced sleep test

Treatment with LQFM032 54 and 162 µmol/Kg decreased sleep latency and increased sleep time (**Figure 3**).

3.4. Open field test

The treatment with LQFM032 at dose of 162 µmol/Kg decreased number of crossings and rearings, while the immobility time was not changed significantly. At the dose of 54 µmol/Kg, LQFM032 did not alter number of crossings and immobility time, but decreased number of rearings. The treatment of different doses of LQFM032 increased the time spent (TCe) and crossings at the central (%CrCe) area (**Table 1**).

3.5. Wire Hanging Test

LQFM032 at differents doses did not alter the values of latency off fall as represented by the group median (**Table 1**).

3.6. Elevated plus maze test (EPM)

The treatments with different doses of LQFM032, increased the percentage of entries and the time spent in open arms (**Figure 4**).

3.7. Light-Dark box test (LDB)

Treatment with LQFM032 at doses of 54 and 162 µmol/Kg increased the number of transitions between the two compartments (light/dark). Moreover, all the doses of LQFM032 increased time spent in light area (**Figure 5**).

3.8. Mechanism of anxiolytic like property

The anxiolytic-like activity of LQFM032 was antagonized by flumazenil. However, pretreatment with NAN-190 did not reversed this activity, demonstrated by number of transitions (**Figure 6A**) and time spent in light area (**Figure 7A**) of LDB.

3.9. Effects of LQFM032 on avoidance memory in stepdown test

LQFM032 (18, 54 and 162 µmol/Kg) did not cause memory impairment on short- and long-term memory (after 90 min and 24 hours, respectively; **Figure 8A**) as demonstrated by an increase in latency to step-down during training session and test sessions. However, when the animals were pre-treatedwith scopolamine 1.0 µmol/Kg, neuroprotective the effect of LQFM032 was reversed (Figura 8B).

3.10. Influence of nicotinic receptor on anxiolytic-like activity of LQFM032

The effect of LQFM032 on the number of transitions (**Figure 9A**) and time spent in light area (**Figure 9B**) of LDB was blocked by mecamylamine.

4.0. Discussion

For preliminary pharmacological screening, gross behavior or Irwin test was carried out. Present findings show that the treatment with LQFM032 induced behavioral alterations such as sedation, ataxia, paralysis of the hindlimbs, that indicate central depressant activity. This test is often used to evaluate the effects of a new compound on physiological and

behavioral functions. Irwin test permits the determination of potential toxicity, appropriate dose and routes of administration. [23, 24, 25]. Moreover, always that possible test is recommended, for detection potential adverse effects of candidate prototypes of drugs on the central nervous system [26]. Based on these results, we choice to administer LQFM032 at 18-162 µmol/Kg p.o. for the pharmacological tests.

Based effects on gross behavior in doses of 18 µmol/Kg, 54 µmol/Kg and 162 µmol/Kg were chosen for evaluation central pharmacological activity of LQFM032. The first test to evaluate specific central activity of LQFM032 was sodium pentobarbital-induced sleep test. This test permits observation of putative CNS depressant and/or stimulant activity because of synergism or antagonism to the depressant activity of sodium pentobarbital [27, 28]. In the present study, the treatment with LQFM032 decreased sleep latency and increase sleep time, thereby suggesting CNS depressant effect. This result is consistent with the previous data on LQFM008 which also demonstrated CNS depressant activity [11].

Compounds with central depressant activity may compromise exploratory activity, which could compromise the results in specific behavioral tests. Hence, open field test was conducted to observe various behavioral parameters, such as rearings and numbers of crossings that indicate exploration activity, grooming number indicating stereotyped activity, among other behaviors including that may suggest an anxiolytic-like activity, which are number of crossings and time spent in the centre of the open field[29, 30]. In the open field test the treatment with LQFM032 (high dose) altered the capacity of locomotion, indicating central depressant activity. Moreover, LQFM032, also at all doses tested, showed anxiolytic-like activity by increased number of crossings and time spent at the center of the open field.

To exclude the possibility that LQFM032 may compromised capacity of motor activity the wire hanging test was employed. In this test, the compound did not alter the fall latency, indicating that the compound did not alter motor activity.

Altogether, the results of sodium pentobarbital-induced sleep test, open field test, and wire hanging test showed the central depressant activity and suggested anxiolytic-like activity of LQFM032. In order to evaluatant the anxiolytic-like activity of this compound, the EPM and LDB tests were conducted. The EPM has been one of the most used behavioral model, that consist of the analysis of the relative time spent in open arms expressed as the percentage and ratio of the number of entries into these arms[31, 32, 33]. In the LDB, parameters such as the number of transitions and the time spent in the light area were used to evaluate anti-anxiety activity of test compound [33]. In these tests, LFQM032 demonstrated anxiolytic-like activity.

In the EPM, the compound LQFM032 increased time spent and entries in the open arms, and increased time spent in the light area and number of transitions in the LDB.

The anxiolytic-like activity of LQFM032 was reversed by flumazenil, benzodiazepine site antagonist [34]. In contrary, NAN-190, 5-HT_{1A} antagonist, did not block the effect of this compound. Hence, these results suggest that the anxiolytic-like activity of LQFM032 is benzodiazepine site dependent. These results are different from the previous data which implicated 5-HT_{1A} receptor in the anxiolytic-like activity of LQFM008 (analog of LQFM032) [11].

Because the anxiolytic effects of classical agonists of benzodiazepines site are associated with undesirable anterograde amnesia [35], it becomes necessary to evaluate the effect of LFQM032 on the performance of experimental animal's memory. In avoidance memory in stepdown test, the treatment with LQFM032 increased the latency to stepdown compared with test session, however the control group showed the same effect. This result suggests that LQFM032 did not improve mmenomic activity in the same way the control group (vehicle), so LQFM032 did not alter this effect.

On the other hand, LQFM032 did not protect the animals of the amnesic effect of scopolamine, muscarinic agonist. Scopolamine produces a impairment in acquisition of new knowledge. The amnesic action produced by scopolamine has been widely used as an experimental model for evaluated drugs with potential cognitive enhancing abilitiy [36].

The LQFM032 showed anxiolytic-like activity without altering mnemonic activity necessitated investigation of the involvement of nicotinic receptor in the anxiolytic-like activity. The pretreatment with mecamylamine antagonist nicotinic [37, 38], reverted the effect of LQFM032 in the LDB by decreasing number of transitions and time in the light area, when compared with group treated with LQFM032.

LQFM032 did not produce a neuroprotective effect when the animals were pre-treated with scopolamine.

The benzodiazepine site dependent effect of LQFM032 without mnemonic impairment can be explained by activation of nicotine acetylcholine receptors that have been reported to activate GABAergic activity. This result suggest that there is a direct relationship between the GABAergic and nicotinic systems whereby acetylcholine activates nicotinic receptors on GABA interneurons in the hippocampus. Whenever the cholinergic pathway is overactivated, the first response is further activation of the GABA interneuron [38, 39]. Nicotinic receptor are known to improve performance on attention and memory tasks [40]. Besides that, nicotinic agonism showed anxiolytic-like activity [41].

Nicotinic agonism effect of LQFM032 can be explained by prototype JWB-1-84-1 which in the structural design of compound LQFM032. JWB-1-84-1 is a choline analog whith nicotinic agonism effect [42]. Previous reports has shown that JWB-1-84-1 is a tertiary amine analogs of choline synthesized with expectation that they would be high potency compounds for cytoprotection, this compound improved cognitive activity[13, 43].

Thereby, the hybridization molecular strategy from LQFM008 (**2**) and JWB-1-84-1 (**3**) allowed to obtain a new piperazine derivative, LQFM032. This new compound showed pharmacology central activity involved benzodiazepine site and nicotinic receptor. These mechanism of action suggested a co-transmission involved nicotinci pathway and modulated benzodiazepine site.

5.0. Conclusion

In summary, LQFM032 showed anxiolytic-like activity that suggest involvement of benzodiazepine site and nicotinic receptors without altering mnemonic activity. Futures studies will be focused on the investigation of possible co-transmission of acetylcholine and GABA, as well as anti-depressant like activity of LQFM032.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Figure 1. **(A)** The structural design of LQFM032 (**4**) from LQFM 008 (**2**) and JWB-1-84-1 (**3**). **(B)** Synthetic route for the preparation of 2-(4-((1-phenyl-1H-pyrazol-4-yl)methyl)piperazin-1-yl)ethanol (**4**) (LQFM032).

Figure 2: (A) Infrared spectrum of 2-(4-((1-phenyl-1*H*-pyrazol-4-yl)methyl)piperazin-1-yl)ethanol (**4**) – LQFM032. (B) ^{1}H NMR expanded spectrum, aromatic region, of -(4-((1-phenyl-1*H*-pyrazol-4-yl)methyl)piperazin-1-yl)ethanol (**4**) – LQFM032 in $CDCl_3$. (C) ^{13}C NMR spectrum of 2-(4-((1-phenyl-1H-pyrazol-4-yl)methyl)piperazin-1-yl)ethanol (**4**) – LQFM032 in $CDCl_3$. (D) Mass spectrum of 2-(4-((1-phenyl-1*H*-pyrazol-4-yl)methyl)piperazin-1-yl)ethanol (**4**) – LQFM032.

Figure 3. Effects of LQFM032, at different doses (p.o), and vehicle 10 mL/Kg (Tween 80 2%) in sodium pentobarbital-induced sleep test as evaluated in mice, (A) latency to (s.) and (B) duration of sleep time (min.). Results are expressed as mean ± SEM; n = 8 each group.

**p≤0.01, ***p≤0.001 – compared with the control group, one-way ANOVA followed by Student-Newnman-Keuls' test.

Figure 4. Effects of LQFM032 at doses of 18, 54 and 162 µmol/Kg or vehicle 10 mL/Kg (Tween 80 2%) on the (**A**) percentage of entries into open arms, and (**B**) time spent in the open arms. Results are expressed as mean ± SEM; n = 8. **p≤0.01, ***p≤0.001 – compared with the control group, one-way ANOVA followed by Student-Newman-Keuls' test.

Figure 5. Effects at differents doses of LQFM032 and control group (vehicle 10 mL/Kg – Tween 80 2%) on (**A**) number of transitions between light and dark compartments, and (**B**) time spent in the light area on LDB. Results are expressed as mean ± SEM; n = 8. **p≤0.01, ***p≤0.001 – compared with the control group, one-way ANOVA followed by Student-Newnman-Keuls' test.

Figure 6. (**A**) Effects of the pre-treatment with saline 0.9%, flumazenil 6.6 µmol/Kg or NAN-190 1.3 µmol/Kg on the effects of LQFM032 54 µmol/Kg in the number of transitions between compartments on LDB. (**B**) Effects of the pre-treatment with saline 0.9% or NAN-190 1.3 µmol/Kg on the effects of buspirone 26 µmol/Kg in the number of transitions on LDB. (**C**) Effects of the pre-treatment with saline 0.9% or flumazenil 6.6 µmol/Kg on the effects of diazepam 3.51 µmol/Kg in the number of transitions on LDB. The same control group (saline + vehicle) was for all figures. Results are expressed as mean ± SEM; n = 8. *p≤0.05, ***p≤0.001 – compared with the control group, and ##p≤0.01, ###p≤ 0.001 – compared with group treated with saline/LQFM032 (**A**), or saline/buspirone (**B**) or saline/diazepam (**C**), one-way ANOVA followed by Student-Newnman-Keuls' test.

Figure 7. (A) Effects of the pre-treatment with saline 0.9%, flumazenil 6.6 µmol/Kg or NAN-190 1.3 µmol/Kg on the effects of LQFM032 54 µmol/Kg on time spent in light area (s) on LDB. (**B**) Effects of the pre-treatment with saline 0.9% or NAN-190 1.3 µmol/Kg on the effects of buspirone 26 µmol/Kg on time spent in light area (s) on LDB. (**C**) Effects of the pre-treatment with saline 0.9% or flumazenil 6.6 µmol/Kg on the effects of diazepam 3.51 µmol/Kg p.o. on time spent in light area (s) on LDB. The same control group (saline + vehicle) was for all figures. Results are expressed as mean ± SEM; n = 8. *p≤0.05, ***p≤0.001 – compared with the control group, and ##p≤0.01, ###p≤ 0.001 – compared with group treated with saline/LQFM032 (A), or saline/buspirone (**B**) or saline/diazepam (**C**), one-way ANOVA followed by Student-Newnman-Keuls' test.

Figure 8. **(A)** Effects at doses of LQFM032 and vehicle (control group – Tween 80 2%) on memory evaluated in the step-down avoidance task, represented by latency to step-down (s). **(B)** Effects of the pre-treatment with vehicle 10 mL/Kg and LQFM032 54 µmol/Kg on the

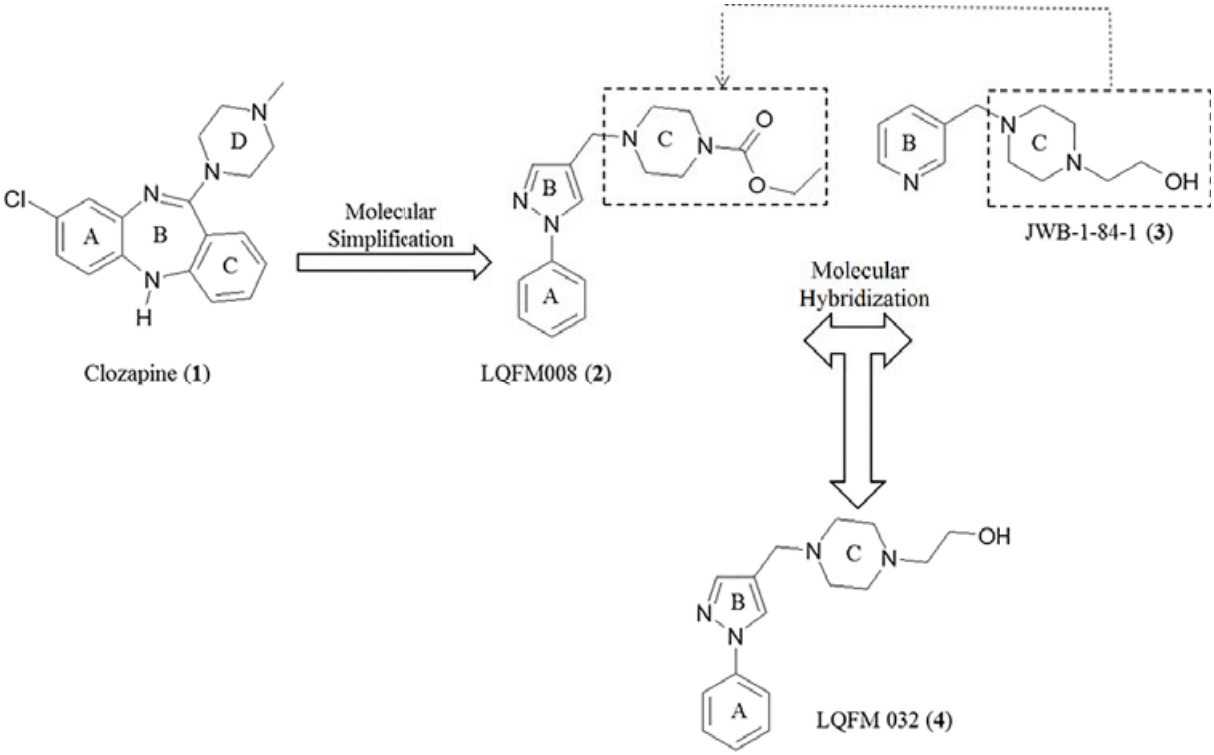
effects of saline 0.9% or scopolamine 1.0 µmol/Kg in memory impairment in mice. TS – training session. Results are expressed as mean ± SEM; n = 8. *p≤0.05, ***p≤0.001 – compared to the training session of the respective group. One-way ANOVA followed by Student-Newnman-Keuls' test.

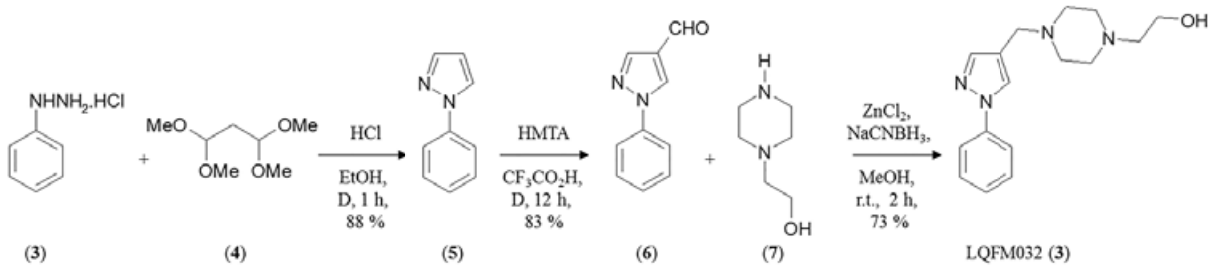
Figure 9. (**A**) Effects of the pre-treatment with saline 0.9% or mecamylamine 30 µmol/Kg on the effects of LQFM032 54 µmol/Kg on number of transitions. (**B**) Effects of the pre-treatment with saline 0.9% or mecamilamine 30 µmol/Kg on the effects of LQFM032 54 µmol/Kg on time spent in light area (s) of light-dark box. Results are expressed as mean ± SEM; n = 8. **p≤0.01 – compared with the control group, and #p≤0.05; ###p≤0.001 – comparison between the groups Mecamilamine+LQFM032 vs Saline+LQFM032.

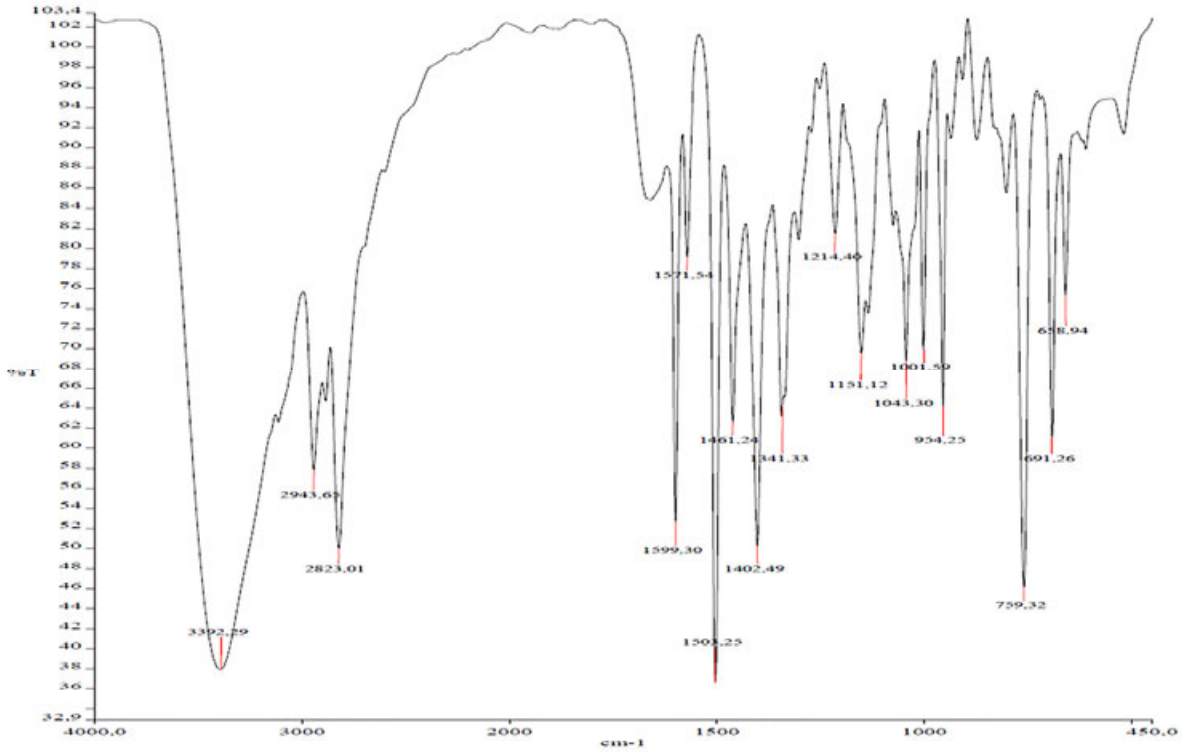
Table 1. Effects of the LQFM032, at different doses, and vehicle 10 mL/Kg (Tween 80 2%) in the open field and wire hanging tests.

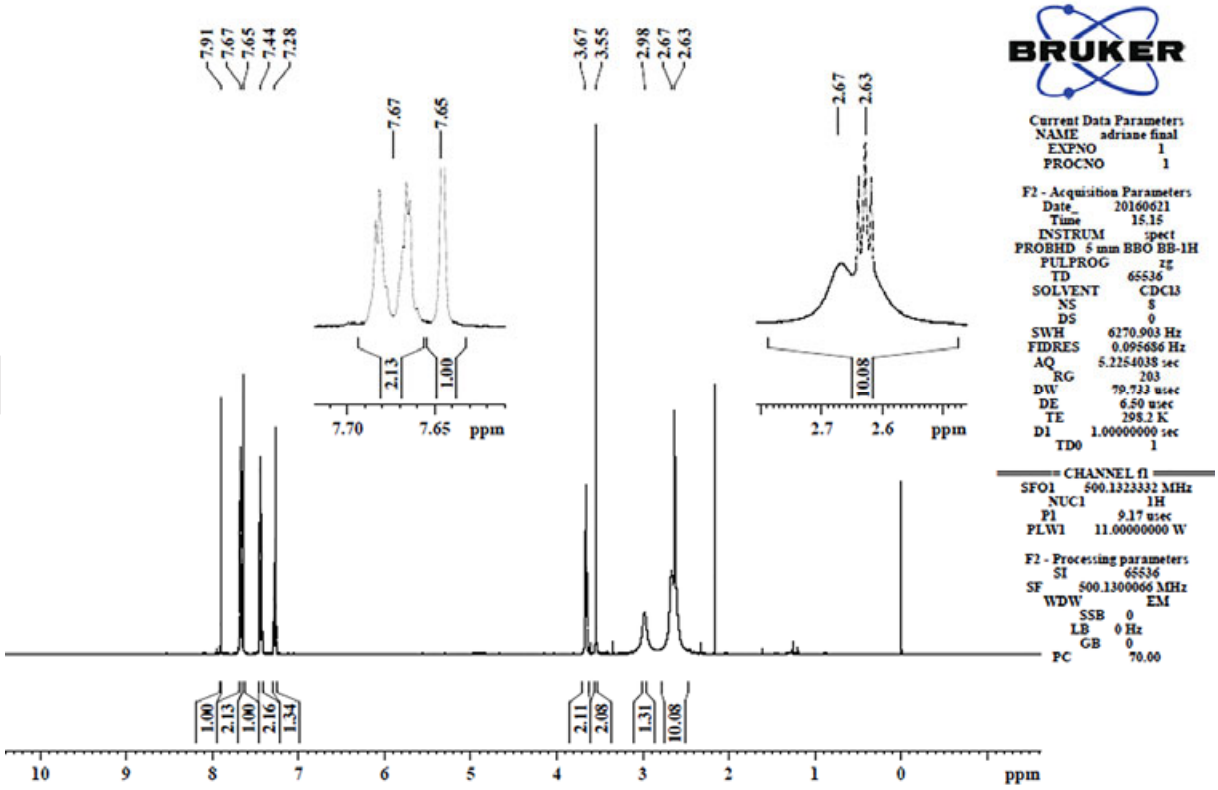
		LQFM032		
	Vehicle 10 ml/Kg	18 µmol/Kg	54 µmol/Kg	162 µmol/Kg
Open Field Test				
Crossings	114.7±1.92	111.7±2.71	106.3±3.07	102.0±2.47**
Rearings	44.29±2.85	43.43±2.82	36.29±2.02*	33.71±1.54*
Grooming	1.00±0.38	1.11±0.31	0.57±0.30	0.50±0.27
Imobility (s.)	1.78±0.36	1.80±0.33	1.67±0.33	2.30±0.30
%CrCe	27.88±1.43	36.63±2.04*	49.14±2.63***	42.29±3.04***
TCe(s)	72.67±1.80	101.70±3.07***	105.3±6.37***	105.7±3.91***
Wire Hanging Test				
Fall latency (s.)	13.67±1.12	14.88±1.32	12.38±0.86	10.63±0.59

Results are expressed as mean ± SEM; n = 8 each group. *p≤0.05, **p≤0.01, ***p≤0.001 – compared with the control group, one-way ANOVA followed by Student-Newnman-Keuls' test. In the wire hanging test, the data are analyzed by the Kruskal-Wallis test followed by Dunns as *post hoc* test and expressed as median (25^{th} percentile to 75^{th} percentile), n=8.









BRUKER
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