Chapter 6

Synthesis and Pharmacological Research Regarding New Compounds with Quinazolin-4-One Structure

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Abstract

The quinazoline scaffold is found in the chemical structure of many marketed drugs used in CNS disorders as antidepressants, anxiolytics, or hypnotics. Also, the carbamate ester derivatives have different certain therapeutic actions, such as hypnotic or parasympathomimetic ones. We have obtained new 4(3H)-quinazolinones by bringing together in the same structure the quinazoline nucleus and carbamate ester group. The compounds named Q1–Q5 were characterized by measuring the melting points, by determining the infrared and NMR spectra, and by elemental analysis. The pharmacological tests evidenced that the compounds have a very low acute toxicity, lethal doses being >2000 mg/kg bw. The compounds had different actions observed in forced swimming test (FST), tail suspension test (TST), or elevated plus maze (EPM), probably influenced by the presence of different radicals on the nucleus. Thus, Q1 with a nitro group in structure manifested the highest antidepressant effect, showing a reduction of immobilization time in FST and TST. On the other hand, Q3 and Q5, with two groups methoxy, respective ethoxy, had a slight anxiolytic effect, highlighted by an increase of the time spent in open arms and a decrease of the time spent in closed arms of EPM.

Keywords: quinazolines, synthesis, antidepressant, anxiolytic, forced swimming test, tail suspension test, elevated plus maze

1. Introduction

The quinazolines constitute an important class of fused heterocycles that are also known as 5,6-benzopyrimidine or benzo[a]pyrimidine, benzo-1,3-diazine, or 1,3-diazanaphthalene. The name quinazoline was first proposed for this compound by Weddige, due to the similarity with cinnoline and quinoxaline [1, 2]. The 4-hydroxyquinazolines, tautomeric with 4-keto-3,4-dihydroquinazolines, are

commonly named 4(3H)-quinazolones; they are an important class of heterocyclic compounds, more than 200 natural compounds having this basic structure [3].

The stability of the quinazolinone nucleus was an important reason why many drug chemistry studies followed synthesis in this class of compounds; a large number of compounds have been synthesized and evaluated for their different biological activities. The first renowned quinazoline marketed drug was methaqualone, used for its sedative-hypnotic effects since 1951 [4].

The quinazoline scaffold is found in the chemical structure of many marketed drugs used in CNS disorders, having antidepressant, hypnotic, and sedative effect (afloqualone, diproqualone, etaqualone, and methaqualone), or used as anticonvulsant (piriqualone), antipyretic, nonsteroidal anti-inflammatory (fluproquazone and proquazone), and antidiabetic agents (balaglitazone, raltitrexed, ispinesib, and halofuginone) [5].

The anticonvulsant action of these compounds has become a priority for pharmacological testing [2], many of these studies highlighting the importance of methyl group at the second position of quinazolin-4(3H)-one [6]. As Gatadi et al. have also shown, more and more recent studies are concerned with investigating the antimicrobial potential of quinazolone derivatives because bacterial strains have developed resistance to available chemotherapeutics [7].

Several research groups have successfully investigated and reported the promising antimicrobial properties and structure-activity relationships (SAR) of various 4(3H)-quinazolinone derivatives [8, 9].

As Hieu et al. reported in recent studies, novel hydroxamic acids incorporating quinazoline-4(3H)-one are a promising class of molecules of interest for the treatment of cancer [10, 11].

On the other hand, it is known that the carbamate ester derivatives have different certain therapeutic actions; this class includes physostigmine, neostigmine, pyridostigmine, rivastigmine, methocarbamol, and carisoprodol.

Carbamates are also of interest for their action as HIV-1 protease inhibitors (darunavir, amprenavir, and atazanavir) [12].

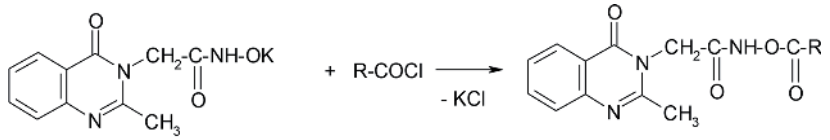
We have concentrated our research activity on bringing together the quinazoline nucleus and carbamate ester group in the same 4(3H)-quinazolinone structure [13].

2. Synthesis of 4(3H)-quinazolinone derivatives

We obtained the new 4(3H)-quinazolone derivatives using the acylation of potassium 2-[2-methyl-3-(4-oxoquinazolin-3(4H)-yl)-aceto]-hydroxamate with aromatic acid chlorides in the presence of dioxane [14]. The general reaction scheme is presented (**Figure 1**).

We obtained the new derivatives by applying the following working technique:

Around 0.68 g (0.0025 mol) of potassium 2-[2-methyl-3-(4-oxoquinazolin-3(4H)-yl)-aceto]-hydroxamate was heating, and then 0.0025 moles of acid chloride in 20 mL of dioxane was gradually added; a white precipitate (potassium chloride)



R= 4-NO_2, 3-CH_3, 3,5-$(OCH_3)_2$, 3,4,5-$(OCH_3)_3$, 3,5-$(OC_2H_5)_2$

Figure 1.
The synthesis of new 4(3H)-quinazolinone derivatives.

is formed. The reaction mixture is refluxed for 3 hours and then filtered. The filtrate was evaporated to dryness by mild heating under vacuum to give the crude product. The new compounds are recrystallized from isopropanol.

All chemicals and solvents were supplied by Sigma-Aldrich Chemical Company. All the solvents were distilled and dried before use.

Melting points were measured in open capillary tubes on an Electrothermal 9100 apparatus, and they are uncorrected.

Infrared spectra were recorded on a FT/IR-solid in ATR spectrometer (the signal intensities (height) were denoted by the following abbreviations: w = weak, m = medium, s = strong, v = variable).

The NMR spectra were recorded on a Varian 2000 and Bruker Fourier 300 instruments at room temperature, operating at 300 MHz for 1H and 75 MHz for 13C. The chemical shifts were recorded in δ units (ppm), relative to residual peak of the deuterated dimethyl sulfoxide (DMSO-d6). Tetramethylsilane (TMS) was used as internal standard. The coupling constants values are reported in hertz, and the splitting patterns are abbreviated as follows: s = singlet, d = doublet, t = triplet, m = multiplet, and b = broad.

The elemental analyses were performed on a PerkinElmer CHNS/O Analyzer Series II 2400 apparatus, and the results were in agreement with the calculated values.

For a better interpretation of spectral data, we used the numbering of the atoms presented in **Table 1**.

Compound Q1: 3-(4-Nitro-phenyl-carbonyl-oxi-carbamoyl-methyl) -2-methylquinazolin-4(3H)-one.

$C_{18}H_{14}N_4O_6$ (Mr 382.33); m.p. 183–184°C; yield 38.7%.

Elemental analysis: Calculated: C 56.55%, H 3.69%, N 14.65%; found: C 56.78%, H 3.56%, N 14.84%.

^{1}H-NMR (dmso-d6, δ ppm, J Hz, T = 308 K): 12.84(s, 1H, H-12); 8.39 (d, 2H, H-16, H-18, J = 9.4); 8.27(d, 2H, H-15, H-16, J = 9.2); 8.11(dd, 1H, H-5, J = 7.8, J = 1.6); 7.82(td, 1H, H-7, J = 7.8, J = 1.6); 7.61(bd, 1H, H-8, J = 7.8); 7.51 (td, 1H, H-6, J = 7.8, J = 1.4); 4.98(s, 2H, H-10); 2.58(s, 3H, H-9).

^{13}C-NMR (dmso-d6, δ ppm, T = 308 K): 164.99(CO-11); 162.69(CO-13); 161.13(C-4); 155.11(C-2); 150.87(C-17); 146.97(C-1a); 131.86(C-14); 119.57(C-4a); 134.71(C-7); 131.13(C-16, C-18); 126.60(C-8); 126.56(C-6); 126.28(C-5); 124.26(C-15, C-19); 43.96(C-10); 22.73(C-9).

FT-IR (solid in ATR, ν cm^{-1}): 3164 m; 3077w; 3017w; 2976 m; 1783s; 1711 m; 1642vs; 1597vs; 1528vs; 1473 m; 1416w; 1386w; 1347s; 1233 m; 1066 m; 975 m; 873w; 844w; 774 m; 710 m; 658w.

Compound Q2: 3-(3-Methyl-phenyl-carbonyl-oxi-carbamoyl-methyl) -2-methylquinazolin-4(3H)-one.

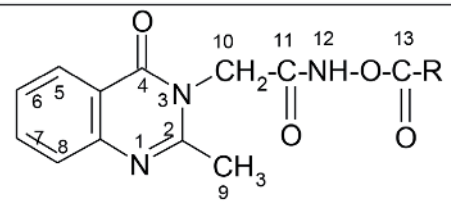
$C_{19}H_{17}N_3O_4$ (Mr 351.36); m.p. 151–52°C, yield 42.5%.

Elemental analysis: Calculated: C 64.95%, H 4.88%, N 11.96%; found 64.80%, H 4.85%, N 12.04%.

^{1}H-NMR (dmso-d6, δ ppm, J Hz, T = 308 K): 12.61(s, 1H, H-12); 8.11(dd, 1H, H-5, 1.4, 8.0); 7.85(t, 1H, H-15,); 7.82(td, H-7, 8.0, 1.4); 7.85÷7.80(m, 2H, H-15, H-19); 7.62(dd, 1H, H-8, 1.4, 8.0); 7.56(bd, 1H, H-17, 7.7); 7.51(td, 1H, H-6, 8.0, 1.4); 7.47(t, 1H, H-18, 7.7); 4.97(s, 2H, H-10); 2.58(s, 3H, H-9); 2.38(s, 3H, H-16′).

^{13}C-NMR (dmso-d6, δ ppm, T = 308 K): 164.86(CO-11); 164.14(CO-13); 161.14(C-4); 155.17(C-2); 147.00(C-1a); 138.78(C-16); 126.42(C-14); 119.61(C-4a);135.18(C-19); 134.70(C-7); 129.85(C-15); 129.10(C-17); 126.73(C-18); 126.60(C-8); 126.55(C-6); 126.30(C-5); 43.94(C-10); 22.73(C-9); 20.73(C-16′).

FT-IR (solid in ATR, ν cm^{-1}): 3200 m; 3004w; 2956w; 1774 m; 1672vs; 1599s; 1519w; 1468 m; 1385 m; 1340w; 1264 m; 1170 m; 1065 m; 972 m; 860w; 778 m; 733 m; 695w.



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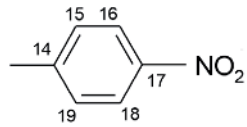
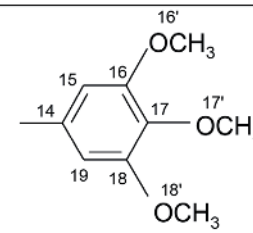
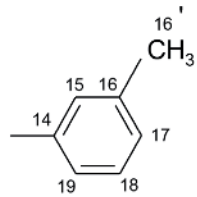
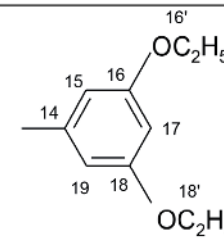
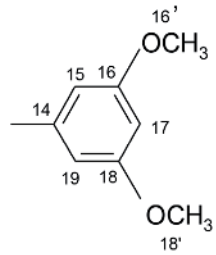
1.		4.	
2.		5.	
3.			

Table 1.
Numbering of atoms for spectral interpretation.

Compound Q3: 3-(3,5-Dimethoxy-phenyl-carbonyl-oxi-carbamoyl-methyl)-2-methylquinazolin-4(3H)-one.

$C_{20}H_{19}N_3O_6$ (Mr 397.39); m.p. 148–49°C, yield 48.1%.

Elemental analysis: Calculated: C 60.45%, H 4.82%, N 10.57%; found C 60.60%, H 4.96%, N 10.65%.

^{1}H-NMR (dmso-d6, δ ppm, J Hz, T = 308 K): 12.58(s, 1H, H-12); 8.10(dd, 1H, H-5, J = 7.8 Hz, J = 1.6 Hz); 7.79(td, 1H, H-7, J = 7.8, J = 1.6); 7.62(bd, 1H, H-8, J = 7.8); 7.51(td, 1H, H-6, J = 7.8, J = 1.5); 7.11(d, 2H, H-15, H-19, J = 2.3); 6.87 (t, 1H, H-17, J = 2.3); 4.96(s, 2H, H-10); 3.81(s, 6H, H-16′, H-18′); 2.58(s, 3H, H-9).

^{13}C-NMR (dmso-d6, δ ppm, T = 308 K): 164.86(CO-11); 163.74(CO-13); 161.13(C-4); 160.69(C-16, C-18); 155.09(C-2); 147.05(C-1a); 128.33(C-14); 119.61(C-4a); 134.65(C-7); 126.63(C-8); 126.51(C-6); 126.26(C-5); 106.99(C-19, C-15); 106.46(C-17); 55.66(C-16′, C-18′); 43.93(C-10); 22.73(C-9).

FT-IR (solid in ATR, ν cm^{-1}): 3239w; 3092w; 2949w; 2844w; 1775 m; 1676vs; 1600vs; 1500w; 1469 m; 1431 m; 1390 m; 1352 m; 1305 m; 1212 m; 1195 m; 1179 m; 1165 m; 1081w; 1051 m; 1016 m; 976 m; 931w; 877w; 848w; 772 m; 747w.

Compound Q4: 3-(3,4,5-Trimethoxy-phenyl-carbonyl-oxi-carbamoyl-methyl)-2-methylquinazolin-4(3H)-one.

$C_{21}H_{21}N_3O_7$ (Mr 427.41); m.p.152–53°C, yield 45.3%.

Elemental analysis: Calculated: C 59.01%, H 4.95%, N 9.83%; found C 58.92%, H 5.05%, N 10.02%.

^{1}H-NMR (dmso-d6, δ ppm, J Hz, T = 308 K): 12.58(s, 1H, H-12); 8.11(dd, 1H, H-5, J = 7.3, J = 1.6); 7.82(td, 1H, H-7, J = 7.3, J = 1.6); 7.62(bd, 1H, H-8, J = 7.3); 7.51(td, 1H, H-6, J = 7.3, J = 1.4); 7.29(s, 2H, H-15, H-19); 4.96(s, 2H, H-10); 3.85 (s, 6H, H-16′, H-18′); 3.76(s, 3H, H-17′); 2.58(s, 3H, H-9).

^{13}C-NMR (dmso-d6, δ ppm, T = 308 K): 164.88(CO-11); 163.64(CO-13); 161.13(C-4); 155.10(C-1a); 152.99(C-16, C-18); 147.05(C-17); 142.69(C-14); 121.30(C-14); 119.61(C-4a); 134.65(C-7); 126.63(C-8); 126.51(C-6); 126.26 (C-5); 106.83(C-15, C-19); 60.25(C-17′); 56.13(C-16′, C-18′); 43.90(C-10); 22.72(C-9).

FT-IR (solid in ATR, ν cm^{-1}): 3200 w; 2979 w; 1766 m; 1680 vs; 1601 s; 1512 m; 1465 m; 1416 wm; 1405 w; 1277 m; 1250 m; 1209 m; 1188 m; 1144 m; 1071 m; 1017 m; 972 m; 870 m; 772 m.

Compound Q5: 3-(3,5-Diethoxy-phenyl-carbonyl-oxi-carbamoyl-methyl)-2-methylquinazolin-4(3H)-one.

$C_{22}H_{23}N_3O_6$ (Mr 425.44); m.p. 158–59°C, yield 35.8%.

Elemental analysis: Calculated: C 62.11%, H 5.45%, N 9.88%; found C 62.25%, H 5.58%, N 10.05%; m.p. 158–159°C; yield 35.8%.

^{1}H-NMR (dmso-d6, δ ppm, J Hz, T = 308 K): 12.59(s, 1H, H-12); 8.11(dd, 1H, H-5, J = 7.8, J = 1.6); 7.82(td, 1H, H-7, J = 7.8, J = 1.6); 7.62(bd, 1H, H-8, J = 7.8); 7.51(td, 1H, H-6, J = 7.8, J = 1.4); 7.07(d, 2H, H-15, H-19, J = 2.1); 6.82(t, 1H, H-17, J = 2.1); 4.96(s, 2H, H-10); 4.07(q, 4H(CH_2), H-16′, H-18′, J = 6.9); 2.58(s, 3H, H-9); 1.32(t, 6H(CH_3), H-16′, H-18′, J = 6.9).

^{13}C-NMR (dmso-d6, δ ppm, T = 308 K): 163.78(CO-11); 163.14(CO-13); 161.09(C-4); 159.88(C-16, C-18); 155.03(C-2); 147.01(C-1a); 128.25(C-14); 119.57(C-4a).

134.58(C-7); 126.58(C-8); 126.44(C-6); 126.20(C-5); 107.30(C-16, C-18); 107.21(C-17); 63.64(CH_2-16′, CH_2-18′); 43.89(C-10); 22.67(C-9); 14.43(CH_3-16′, CH_3-18′).

FT-IR (solid in ATR, ν cm^{-1}): 3219 w; 2980 w; 2937 w; 2882 w; 1778 m; 1675 vs; 1602 s; 1510 w; 1471 w; 1451 m; 1389 m; 1372 m; 1355 m; 1301 m; 1179 vs; 1116 m; 1086 m; 1058 s; 976 m; 934 m; 859 m; 829 w; 776 m; 747 m; 708 w; 692 w; 676 w; 658 m.

3. Pharmacological research on new 4(3H)-quinazolinone derivatives

3.1 Objective

The main objective of our study was to assess the potential pharmacological actions on central nervous system of five new 4(3H)-quinazolinone derivatives. For this purpose, we evaluated first the acute oral toxicity on mice, using the "up and down" method, in accordance with European Guidelines regarding the ethic of experimental research on animals [15]. These guides mention that the substances expected to have a low degree of toxicity can be tested using the limit test

at 2000 mg/kg bw and only in special situations at the dose of 5000 mg/kg bw. After assessing the toxicity level of the substances, we performed a battery of tests to highlight the pharmacological potential of the new compounds on the central nervous system. Thus, we determined the antidepressant effect using the forced swimming test (FST) and tail suspension test (TST); the effect on anxiety using the elevated plus maze (EPM), Ugo Basile, Italy; and the effect on the motor activity with the activity cage (Ugo Basile).

All pharmacological tests were performed on mice, following all the existing protocols from the Laboratory of Pharmacology, Faculty of Pharmacy, UMF "Carol Davila" Bucharest.

3.2 Materials and methods

We used for the pharmacological experiments 85 male, white, NMRI mice, weighing 26 ± 1.7 g. The animals were supplied by the rodent farm of the University of Medicine and Pharmacy "Carol Davila" Bucharest. The animals were housed in ventilated cages with free access to food and water. The temperature and the relative humidity were kept constant (22–24°C, 45–60%).

All experimental procedures were carried out in accordance with the Directive 2010/63/UE of 22 September 2010, regarding the protection of animals used for experimental and other scientific purposes. All experimental procedures were approved by the Ethical Committee of the Faculty of Pharmacy, Bucharest. The experiment was conducted in May 2018.

For acute toxicity evaluation, we used five groups of three mice each, which received the new five quinazolinone derivatives in the dose of 2000 mg/kg bw p.o. All animals were followed for 14 days regarding any sign of lethality, body weight evolution, or behavior changes.

The pharmacological tests on central nervous system were performed on 70 mice, which were initially subjected to the Ugo Basile activity cage test. The parameter on which the animals were divided into groups was the horizontal motor activity, measured for every period of 5 min. The mice were divided into 7 groups of 10 individuals each, with similar average responses and standard deviations between groups.

The compounds were administered as shown below:

- Group I (control)—distilled water 0.1 ml/10 g orally
- Group II (reference)—amitriptyline 10 mg/kg bw susp. 0.1% orally
- Group III—Q1 100 mg/kg bw, susp. 1% orally
- Group IV—Q2 100 mg/kg bw, susp. 1% orally
- Group V—Q3 100 mg/kg bw, susp. 1% orally
- Group VI—Q4 100 mg/kg bw, susp. 1% orally
- Group VII—Q5 100 mg/kg bw, susp. 1% orally

We decided to test the animals at a dose of 100 mg/kg bw, considering the level of 1/20 of the dose administered in acute toxicity test which provided no lethal effects to mice.

The pharmacological tests were performed as follows:

- After 1 day of administration: activity cage and FST
- After 14 days of administration: activity cage and TST
- After 15 days of administration: EPM

FST was chosen after acute administration because it proved effective and consistent in testing antidepressant effect after one single dose [16].

All tests were conducted respecting the following protocol: in the testing chamber, the animals were kept in artificial light. Each individual was administered with a 7-min delay from the previous one (5 min for the test itself and 2 min to clean the device before testing the next animal) so that all of them could be tested after the same time interval from the moment of receiving the treatment.

Determination of motor activity assessed the influence of new compounds on mice motility and desire to explore. The duration of this test in the activity cage was 5 min for each mouse. The animals were placed each time in the same corner of the device [17].

Determination of immobility time of mice in forced swimming test (*FST*), was originally described by Porsolt [16, 18]. Each mouse was placed into a glass cylinder (25 cm height, 30 cm diameter) containing water at a temperature of 23 ± 1°C. The test duration was 6 min, the first two for accommodation and the next four for the actual determination of the immobilization time. The mouse is considered immobilized when it ceases to struggle and remains in an immobile, characteristic position, with minimal movements for keeping the head above the water.

Determination of immobility time of mice in tail suspension test (TST) involves the same principle as FST, the difference being the nature of the inducing factor of the depressive state, the suspension of the animal by the tail. In this test, there is no need for accommodation, so the determination of the immobility time starts from the beginning of the experiment [19].

Determination of anxiolytic potential of the compounds was performed using the *elevated plus maze Ugo Basile*. The mouse was placed in the center of the device, and it was left free to explore the maze. We determined the time spent into the open arms and the time spent into the closed arms. We considered that the mouse was in one of the arms when all four limbs were in that arm [20].

3.2.1 Statistical analysis

Statistical calculation used the software GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com. Statistical comparison between groups used the ANOVA test. In case it indicated a statistical significance, the Tukey posttest was performed comparing all groups 2 × 2. When appropriate, we determined the Pearson correlation coefficient.

Normality of response distribution in collectivity was tested with D'Agostino and Pearson test.

3.3 Results and discussion

Acute toxicity research did not lead to any lethality for the five new compounds administered at a dose of 2000 mg/kg bw. According to the "up and down" method, higher doses are not recommended, and the compounds can be classified in the

	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
M	623.2	622.1	623.5	622.6	623.0	622.8	623.9
SD	100.7	104.1	86.1	107.7	95.25	99.29	107.52

M = average, SD = standard deviation.

Table 2.
The horizontal motor activity (HMA) of the groups formed after the selection process.

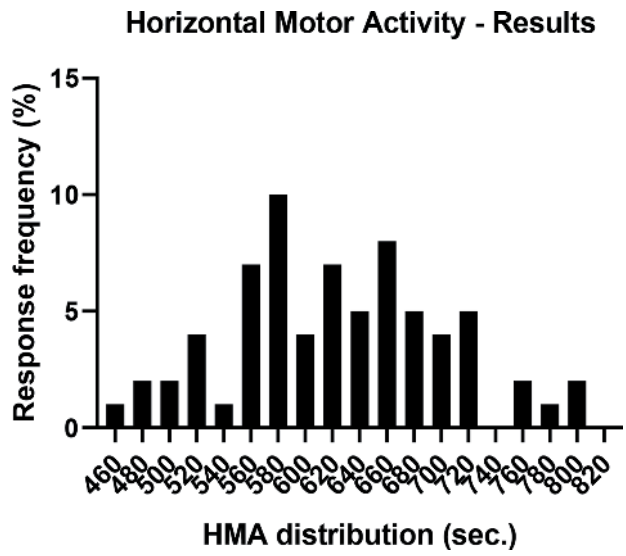


Figure 2.
Normal distribution of initial HMA values.

category "very low toxicity." Evolution of body weight, determined every other day for 14 days, was similar between treated mice and control ones. Changes in weight were small, being statistically insignificant. Motor behavior was similar, and response to auditory and tactile stimuli was present. No animal showed any palpebral ptosis, and the appearance of the fur and tail remained unchanged during the experiment.

After the *initial determination of motor activity*, the mice were divided into seven homogenous groups, each containing 10 individuals. Their baseline mean horizontal motor activity is shown in **Table 2**, and the Gaussian distribution of the individual results is highlighted in **Figure 2**:

After acute administration, compound Q5 had the most intense effect on motor activity, with a 23.69% reduction in HMA compared with the control group. This result was statistically significant according to ANOVA followed by Tukey posttest. The same compound reduced VMA with 37.39% compared with the control, but this result was not significant. Q3 was another compound which reduced the motor activity by more than 20%, but the results were not significant. The other three new quinazolinones had limited influence on motor activity. Amitriptyline used as the reference substance did not influence significantly the motor activity after one administration, as it can be observed in **Tables 3** and **4**.

The immobilization time in FST after acute administration was influenced differently by the new five compounds and was correlated with the results obtained in motor activity testing. In **Table 5**, it can be seen that compounds that have decreased the most intense motor activity (Q3, Q5) have led to an increase in immobilization time, with 44.09 and 41.24% compared with the reference group. Compound Q1 had the most intense antidepressant effect after one dose, quantified by reducing the immobilization time with 20.39% compared with the control group.

	M ± SD	ANOVA	Effect % vs. control	Tukey posttest/ control	Effect % vs. reference	Tukey posttest/ reference
Group I (control)	500.50 ± 91.25	<0.001**	—	—	—	—
Group II (reference)	477.50 ± 80.27		−4.59%	ns	—	—
Group III (Q1)	499.00 ± 46.82		−0.29%	ns	−4.50%	ns
Group IV (Q2)	472.50 ± 57.33		−5.59%	ns	−1.05%	ns
Group V (Q3)	398.50 ± 52.26		−20.37%	ns	−16.54%	ns
Group VI (Q4)	436.50 ± 107.1		−12.78%	ns	−8.58%	ns
Group VII (Q5)	381.90 ± 107.0		−23.69%	<0.05*	−20.02%	ns

*Statistical significance
**High statistical significance

Table 3.
The horizontal motor activity (HMA) of the groups after one administration.

	M ± SD	ANOVA	Effect % vs. control	Effect % vs. reference
Group I (control)	59.90 ± 26.94	ns	—	—
Group II (reference)	50.90 ± 15.59		−15.02%	—
Group III (Q1)	50.70 ± 14.21		−15.35%	−0.39%
Group IV (Q2)	52.50 ± 19.73		−12.35%	−3.14%
Group V (Q3)	45.50 ± 15.38		−24.04%	−10.60%
Group VI (Q4)	46.30 ± 17.01		−22.70%	−9.03%
Group VII (Q5)	37.50 ± 28.16		−37.39%	−26.33%

Table 4.
The vertical motor activity (VMA) of the groups after one administration.

	M ± SD	ANOVA	Effect % vs. control	Tukey posttest/ control	Effect % vs. reference	Tukey posttest/ reference
Group I (control)	121.10 ± 34.51	<0.0001***	—	—	—	—
Group II (reference)	98.20 ± 18.97		−18.91%	ns	—	—
Group III (Q1)	96.40 ± 14.47		−20.39%	ns	−1.83%	ns
Group IV (Q2)	101.30 ± 16.40		−16.35%	ns	3.15%	ns
Group V (Q3)	138.70 ± 28.13		14.53%	ns	41.24%	<0.01**
Group VI (Q4)	132.70 ± 26.03		9.57%	ns	35.13%	<0.05*
Group VII (Q5)	141.50 ± 27.73		16.84%	ns	44.09%	<0.01**

*Statistical significance.
**High statistical significance.
***Statistical significance. Very high statistical significance.

Table 5.
The immobilization time in FST after one administration.

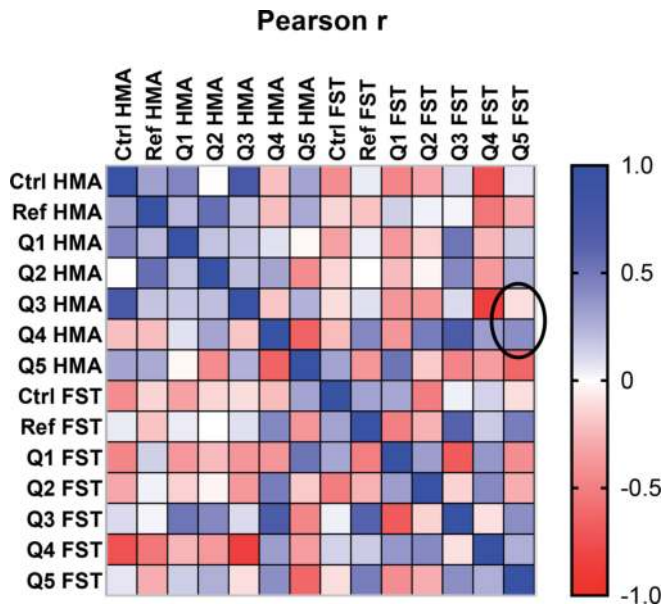


Figure 3.
Pearson correlation between data obtained in activity cage test and forced swimming test after acute administration (black circle—the best correlation for a compound between immobilization time in FST and horizontal movements activity).

	M ± SD	ANOVA	Effect % vs. control	Tukey posttest/ control	Effect % vs. reference	Tukey posttest/ reference
Group I (control)	471.50 ± 72.75	<0.0001***	—	—	—	—
Group II (reference)	382.90 ± 64.70		−18.79%	<0.05*	—	—
Group III (Q1)	479.50 ± 65.91		1.69%	ns	25.22%	<0.05*
Group IV (Q2)	482.50 ± 72.71		2.33%	ns	26.01%	<0.05*
Group V (Q3)	404.30 ± 50.62		−14.25%	ns	5.58%	ns
Group VI (Q4)	468.30 ± 56.68		−0.67%	ns	22.30%	ns
Group VII (Q5)	365.50 ± 52.63		−22.48%	<0.01**	−4.54%	ns

*Statistical significance.
**High statistical significance.
***Very high statistical significance.

Table 6.
The horizontal motor activity (HMA) of the groups after 14 days of administration.

It was interesting to find the degree of Pearson correlation between the values obtained in the two tests after acute administration. As it can be observed in **Figure 3**, the best correlation of data is between values of group Q5—HMA and FST—with a coefficient of −0.622.

After 2 weeks of daily administration, the motor activity of the mice has illustrated a significant reduction of HMA in Q5 group, with 22.48% compared with control, which can be seen in **Table 6**. Also, in accordance with the known fact that amitriptyline has a sedative pharmacological profile, it determined a marked decrease of horizontal movements, with 18.79% compared with control.

Regarding the vertical movements, amitriptyline and the new compounds Q3 and Q5 produced the largest decrease, with effects between 14.63 and 34.03%, but ANOVA showed no statistical significance (**Table 7**).

	M ± SD	ANOVA	Effect % vs. control	Effect % vs. reference
Group I (control)	56.70 ± 17.13	ns	—	—
Group II (reference)	47.40 ± 16.53		−16.40%	—
Group III (Q1)	59.00 ± 14.79		4.05%	24.47%
Group IV (Q2)	51.10 ± 17.99		−9.87%	7.80%
Group V (Q3)	48.40 ± 10.99		−14.63%	2.10%
Group VI (Q4)	51.80 ± 14.48		−8.64%	9.28%
Group VII (Q5)	37.40 ± 15.58		−34.03%	−21.09%

Table 7.
The vertical motor activity (VMA) of the groups after 14 days of administration.

	M ± SD	ANOVA	Effect % vs. control	Tukey posttest/ control	Effect % vs. reference	Tukey posttest/ reference
Group I (control)	103.80 ± 14.81	<0.01**	—	—	—	—
Group II (reference)	83.60 ± 14.67		−19.46%	ns	—	—
Group III (Q1)	82.90 ± 12.16		−20.13%	ns	−0.83%	ns
Group IV (Q2)	94.10 ± 14.62		−9.34%	ns	12.55%	ns
Group V (Q3)	104.20 ± 18.61		0.38%	ns	24.64%	ns
Group VI (Q4)	96.20 ± 19.86		−7.32%	ns	15.07%	ns
Group VII (Q5)	107.60 ± 22.46		3.66%	ns	28.70%	<0.05*

**High statistical significance Statistical significance*
***Statistical significance.*

Table 8.
The immobilization time in TST after 14 days of administration.

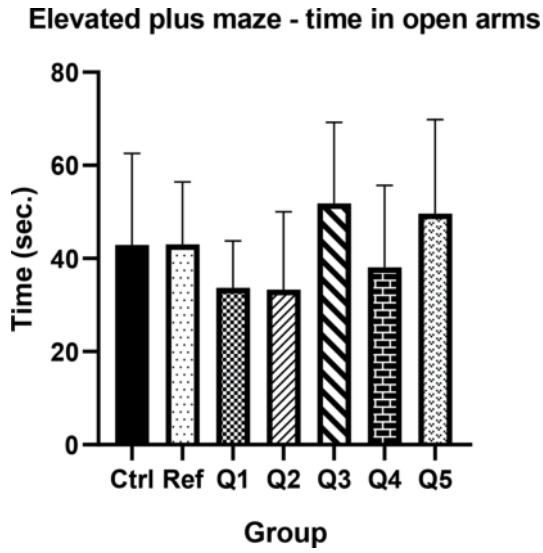


Figure 4.
Time in open arms of EPM after 15 days of administration (the columns represent the mean + SD).

Tail suspension test after 14 days of administration evidenced the compound Q1 which reduced the immobilization time with 20.13% compared with control. The effect is comparable to that of the reference substance. Compound Q5 produced an increase of immobilization time, with 3.66% compared with control and 28.70% compared with amitriptyline (**Table 8**).

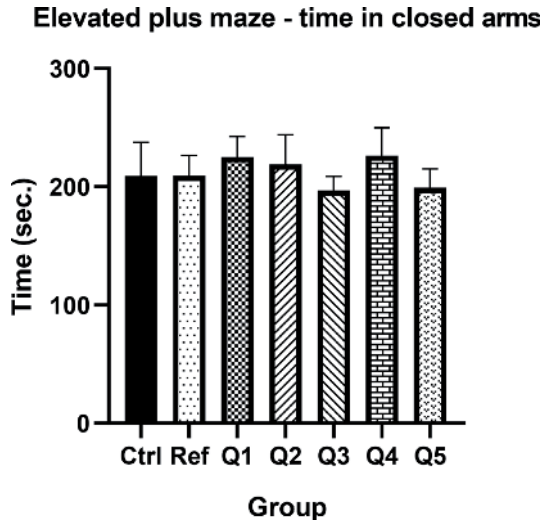


Figure 5.
Time in closed arms of EPM after 15 days of administration (the columns represent the mean + SD).

At the end of the experiment, we wanted to see if the new quinazolinones had any influence on the anxious natural behavior of the mice. It is well known that placed in the plus maze, mice prefer to explore closed and secure arms instead of open ones, associated with imminent danger. The results in elevated plus maze test evidenced a slight anxiolytic effect for two new compounds, Q3 and Q5. They increased the time spent in the open arms of the maze with 20.74 and 15.61% compared with control group. The mean results can be observed in **Figure 4**.

Also, the same two compounds produced a decrease of the time spent in closed arms of the maze, with 5.92 and 4.92% compared with control (**Figure 5**).

The preference of the animals for the open arms instead of closed ones is a sign of lower anxiety, thus we can affirm that compounds Q3 and Q5 have a slight anxiolytic effect. The other compounds did not influence the parameters in EPM.

4. Conclusion

We obtained five new 4(3H)-quinazolinone derivatives through a standardized synthesis process. The compounds were characterized by measuring the melting points, by determining the infrared and NMR spectra, and by elemental analysis.

The pharmacological tests evidenced that the five new quinazolinones have a very low acute toxicity, lethal doses being >2000 mg/kg bw.

Regarding the results obtained in pharmacological tests for evaluation of antidepressant and anxiolytic effects, the compounds had different actions, probably influenced by the presence of different radicals on the nucleus.

Thus, Q1 which have the nitro group in structure manifested the highest antidepressant effect, with a reduction of immobilization time in FST with 20.39% and in TST with 20.13% compared with control.

On the other hand, compounds Q3 and Q5, with two groups methoxy, respective ethoxy, had a slight anxiolytic effect, highlighted by increasing the time spent in open arms, with 20.74 and 15.61% compared with control.

The five new compounds have been shown to have central nervous system activity, and we consider that they deserve further testing in order to detect other effects of interest.

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Conflict of interest

The authors declare no conflict of interest.

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