

Synthesis and study of the effect of 3-substituted chromone derivatives on changes in the activity of mitochondrial complex III under experimental cerebral ischemia

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ABSTRACT: Chromone derivatives are characterized by an extensive spectrum of pharmacological activity and can be potentially effective neuroprotective agents. In this regard, this study evaluated the effect of eighteen new derivatives of 3-formylchromone on the change in the actinic activity of mitochondrial complex III in ischemic and intact animals as a promising direction of neuroprotective therapy. The dependence of the level of pharmacological effect on changes in the quantum-chemical parameters (Mulliken Charge, Bond number, Theoretical valence, Unsaturated index, Electron density, Free valence index) of the molecules was also evaluated. The structure of the substances was confirmed by IR, UV and NMR spectroscopy, as well as by determining the melting point. The study made it possible to establish that, among the 3-substituted chromone derivatives, the most pronounced neuroprotective activity is possessed by compounds that do not contain halogen atoms in their structure. The leader compound can be considered 3 - [(E) -3-(3,5-di-tert-butyl-4-hydroxy-phenyl) -3-oxo-prop-1-enyl] -6-methoxy-chromen-4-one, the use of which increased the activity of mitochondrial complex III by 85.2% ($p < 0.05$) in relation to the negative control group. To the greatest extent, the influence of the test substances on the activity of the mitochondrial complex III depended on the change of Free valence index (correlation coefficient, $r=0,93701$)

KEYWORDS: Substituted chromone derivatives; mitochondrial complex III; neuroprotection; mitochondrial dysfunction

1. INTRODUCTION

Mitochondria are cell organelles that perform three main functions in the cell: generating ATP and maintaining energy homeostasis; regulation of the production of reactive forms of oxygen (ROS) as molecules that control epigenetic processes; control of apoptosis reactions, both external and internal cascade [1]. The leading organizational component of the mitochondrial matrix is the electron transport chain (ETC). The ETC consists of four protein supercomplexes (I-IV) localized on the inner mitochondrial membrane. Initially, complexes I and II oxidize NADH and FADH₂, respectively, transferring the obtained electrons to ubiquinol, which transfers electrons to complex III. Complex III, in turn, transports electrons through the intermembrane space to cytochrome C, and, accordingly, to complex IV. Then complex IV uses electrons to reduce oxygen to water [2]. In the course of electron transport reactions, the generation of ROS is observed, and their leakage from the ETC can be noted, which leads to the initiation of oxidative stress. Complexes I and II generate ROS (mainly represented by superoxide radical) in the mitochondrial matrix, while complex III generates ROS both in the matrix and in the intermembrane space, which makes this organizational component of the ETC the leading site of ROS formation [3].

Complex III generates ROS during Q-cycle reactions (successive oxidation and reduction reactions of CoQ). In this process, ubisemiquinone in a non-enzymatic reaction can freely give an unpaired electron to oxygen, with the formation of ROS (superoxide radical). In turn, ROS are able to be released into the

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mitochondrial matrix and penetrate through the mitochondrial inner membrane. The process of ROS penetration through the inner membrane of mitochondria proceeds by two mechanisms: with the catalytic conversion of a superoxide radical into a peroxide—a more stable molecule, or with the participation of anionic mitochondrial channels [4].

Thus, a targeted pharmacological effect on mitochondrial complex III may be a promising direction in the treatment of oxidative stress and associated diseases, for example, ischemic stroke [5].

Chromone is known to be benzannulated 4H-pyranone-4. Chromone derivatives constitute one of the most numerous groups of natural compounds, of which the most studied derivatives are 2-arylchromone-flavones and 3-arylchromone-isoflavones. Representatives of these compounds are included in the main classes of flavonoids and have more than 5000 described structures, characterized by about 50 types of pharmacological activity [6]. It has now been established that the chromone nucleus is a promising basic structure for the targeted synthesis of new compounds with desired pharmacological properties. In particular, chromone derivatives are used in the field of neurodegenerative, inflammatory and infectious pathologies, as well as diabetes mellitus and cancer [7]. In addition, the antiallergic properties of chromone derivatives are widely known, which are associated with the stabilization of mast cells cell membranes [8]. In an earlier study, chromone derivatives had a positive effect on changes in mitochondrial function, normalizing the reactions of aerobic/anaerobic metabolism, which served as the basis for choosing representatives of a number of derivatives of 3-formylchromone as the test-objects [9].

Considering also that the structural diversity of the types, number and position of substituents attached to the main chromone nucleus is important for a directed change in the physical, chemical and pharmacological properties of the target compounds [10], we carried out this work.

2. RESULTS

2.1 Results of assessing the effect of the studied compounds on the change in the activity of mitochondrial complex III

The effect of the test-compounds on the activity of the mitochondrial complex III was assessed in ischemic and intact animals (Table 1). As a result, it was found that the administration of the test-substances to intact animals did not have a significant effect on changes in the activity of ubiquinolcytochrome-c-oxidoreductase. At the same time, in rats with cerebral ischemia, which did not receive pharmacological support, a decrease in the activity of this respiratory complex by 52% ($p < 0.05$) was noted in relation to SO (sham-operated) animals (Table 1). On the background of the administration of the CoQ10, the catalytic properties of ubiquinolcytochrome-c-oxidoreductase in comparison with the NC (negative control) group of rats increase by 39% ($p < 0.05$). The use of test compounds C3ACL; C3AF; C3AI; C3A6Ac and C3ACLNOH; C3AACP1; C3AACP2; C3AACP3; C3AACP4;; C3AACP5 C3AACP6; C3AACP7 also increased the activity of mitochondrial complex III by 42.3%; 64.2%; 30.9%; 25.2%; 26%; 31.7%; 43.9%; 67.5%; 53.7%; 41.5%; 46.3%; 60.2% and 85.2% respectively (all indicators $p < 0.05$ relative to the NC group of rats). At the same time, the activity of ubiquinolcytochrome-c-oxidoreductase in animals that received compounds C3AACP2 and C3AACP7 was higher than that in rats that received CoQ10 (Table 2) by 20.4% ($p < 0.05$) and 33.3% ($p < 0.05$) respectively.

Table 1. Influence of the test compounds and the reference drug on the change in the activity of the mitochondrial complex III in the brain tissue in rats against the background of focal cerebral ischemia and in intact animals

Group	Intact animals	Ischemia
SO	24.8±1.572	25.6±2.225
NC	-	12.3±1.276#
CoQ ₁₀	24.2±1.895	17.1±2.343*
C3A	24.3±2.56	14.4±2.398*
C3ACL	23.7±2.894	17.5±1.817*
C3AF	22.7±1.851	20.2±2.535*
C3AI	23.9±2.687	19.9±2.45*
C3A6Ac	25.7±2.098	12.4±2.635
C3A7Ac	24.9±1.68	14.2±2.541
C3AOCH ₃	24.7±1.246	12.2±2.624
C3ACH ₃ Phen	25.9±2.053	1.1±1.108
C3ANOH	24.6±2.867	13.4±1.908
C3AFNOH	23.6±2.249	15.5±1.562*
C3ACLNOH	22.5±2.038	16.2±2.747*
C3AACP1	24.3±2.4	17.7±1.291*
C3AACP2	25.1±2.217	20.6±2.09* Δ
C3AACP3	24.5±2.986	18.9±1.063*
C3AACP4	24.2±2.639	17.4±2.589*
C3AACP5	25.3±2.854	18±1.564*
C3AACP6	23.3±2.417	19.7±2.868*
C3AACP7	24.7±2.635	22.8±1.687* Δ

Note: SO - sham-operated rats; NC- negative control rats; # - significantly relative to the SO animals (Newman-Keuls test, p <0.05); * - significantly relative to the NC group of animals (Newman-Keuls test, p <0.05); Δ - significantly relative to the group of animals treated with CoQ₁₀ (Newman-Keuls test, p <0.05).

2.2. Correlation analysis results

Table 2 shows the summary quantum-chemical parameters used in the correlation analysis.

The data of the pharmacological experiment showed a significant difference in the effect of compounds that do not contain halogens compared to that of halogen-containing derivatives. In this regard, in the correlation analysis, both subgroups of test-compounds was carried out (Table 3).

Table 2. Total values of quantum chemical parameters

Compound	Mulliken Charges, (a.u.)	Bond numbers (N μ)	Theoretical valence (V μ)	Unsaturated index (IUA)	Electron density (ED)	Free valence index (F μ)
C3A	-0.96	43.662	45.695	2.033	58.9557	9.531
C3ACL	-1.26	49.269	51.558	2.289	69.2631	10.68
C3AF	-1.4	54.773	57.575	2.802	79.4021	11.67
C3AI	-1.43	54.592	57.437	2.845	79.4296	11.843
C3A6Ac	-0.843	44.591	46.768	2.177	65.8429	5.598
C3A7Ac	-0.841	44.62	46.816	2.196	65.8411	9.577
C3AOCH3	-0.83	44.649	46.798	2.149	65.8298	9.564
C3ACH3Phen	-2.127	72.609	75.968	3.359	93.1269	16.452
C3ANOH	-1.33	46.663	48.8	2.14	64.3331	9.421
C3AFNOH	-1.22	46.588	49.873	3.285	71.2223	9.492
C3ACLNOH	-1.22	47.632	49.919	2.287	71.2211	9.475
C3AACP1	-2.01	74.135	77.33	3.195	92.0069	16.737
C3AACP2	-2.66	81.896	85.212	3.316	100.656	18.435
C3AACP3	-2.56	79.519	83.11	3.591	94.3222	17.882
C3AACP4	2.0561	78.8170	80.4190	1.6020	105.0559	17.027
C3AACP5	-2.3471	80.7590	84.3970	3.6380	112.3472	17.838
C3AACP6	-2.48	81.478	85.155	3.677	108.4686	18.149
C3AACP7	-4.86	112.744	116.857	4.113	142.8623	24.624

From the data in the table 3, it follows that the correlation coefficients for compounds that do not contain halogens are significantly higher than for halogenated derivatives. The maximum values of r are observed when analyzing such parameters as the free valence index (F μ), electron density, and unsaturation index (IUA). Thus, when studying the structure - activity relationship, the most reliable results are achieved for chromone derivatives that do not contain halogens, and the F μ parameters and electron density are used in the correlation equations.

Table 3. The value of the correlation coefficients

With halogen-containing compounds		Without halogen-containing compounds	
Parameter	r	Parameter	r
a.u.	0.3737	a.e.	0.7877
N μ	0.74010	N μ	0.78655
V μ	0.73927	V μ	0.78952
IUA	0.51634	IUA	0.84437
ED	0.77884	ED.	0.86929
F μ	0.76814	F μ	0.93701

Note: a.u - Mulliken Charges; N μ - Bond numbers; V μ - Theoretical valence; IUA - Unsaturated index; ED- Electron density;F μ - Free valence index.

3. DISCUSSION

Globally, ischemic stroke is the second leading cause of death and the first cause of disability in the adult population. The «gold standard» in the treatment of this pathological condition is the fastest possible recanalization of the vessel, achieved by intravenous administration of tissue plasminogen activator (t-PA). Nevertheless, this pharmacotherapeutic approach has drawbacks, which include a small «therapeutic window» and the development of post-reperfusion complications [11]. In this regard, a number of attempts to improve reperfusion treatment have been made, including through the use of adjuvants, for example, neuroprotective agents, which are currently being actively studied by the world scientific community [12]. The main direction of the action of neuroprotectors is the elimination of pathogenetic pathways of neuronal damage during ischemia, for example, oxidative stress, the activity of which directly depends on the adequate course of electron transport reactions in the mitochondrial ETC [13].

The mitochondrial respiratory chain consists of four multimeric complexes that catalyze redox reactions, with the participation of NADH and FADH₂ as electron donors, which ultimately reduces molecular oxygen to water. This electron transfer is associated with the formation of a proton electrochemical gradient required for the condensation of ADP and phosphate, and, accordingly, the formation of ATP. Complex III or ubiquinolcytochrome-c-oxidoreductase is the center of the respiratory chain, transferring electrons from coenzyme Q to cytochrome C [14]. Dysfunction of complex III leads to the development of irreversible metabolic disorders that mediate a high degree of cellular damage. Thus, when the catalytic reactions of complex III are suppressed, there is overproduction of free radicals, a decrease in ATP synthesis, the formation of a mitochondrial pore of transient permeability with an increased influx of ionized calcium and cell death as a result of apoptosis or pyroptosis [15]. In this regard, the dysfunction of complex III has an extremely negative effect on the state of organs with high metabolic activity, for example, the brain. At the same time, suppression of the physiological activity of ubiquinolcytochrome c-oxidoreductase is undesirable in view of the catastrophic drop in ATP synthesis in cells with normal metabolism [16].

The study showed that the use of newly synthesized 3-substituted chromone derivatives at a dose of 40 mg / kg *per os* increases the activity of mitochondrial complex III in the brain of animals with reproduced cerebral ischemia, but does not change the activity of this supercomplex in rats without pathological background, which can be evidence of the regulatory action of 3-substituted chromone derivatives in relation to ubiquinolcytochrome-c-oxidoreductase. At the same time, a regularity was noted that the inclusion of halogen atoms in the target structure significantly worsens the pharmacological effect. In this connection, a correlation analysis was carried out of the dependence of the regulatory capabilities of the studied compounds in relation to complex III and quantum-chemical parameters that most fully characterize the features of the molecular structure. This analysis showed that, with a high degree of probability, halogen atoms worsen the pharmacotherapeutic response to the administration of 3-substituted chromone derivatives, with its consistently high indicators in a number of substances that do not contain halogens in their structure. At the same time, the activity of the compounds under study largely depends on the value of the free valence index ($r = 0.93701$). Based on the F_{μ} value, one can draw conclusions about the reactivity of molecules containing π -bonds: the larger the F_{μ} value, the higher the probability for the attachment of neutral particles, which is most important for the mitochondria-oriented action of pharmacologically active substances [17]. At the same time, it is known that halogen atoms, being in the aromatic nucleus, have a strong negative inductive effect (-I effect), clearly prevailing over a weak positive mesomeric effect (+ M effect), i.e. $-I \gg + M$, which probably distorts the π -electron cloud and leads to a decrease in therapeutic efficacy [18].

4. CONCLUSION

The study showed that among the studied 3-substituted chromone derivatives, the highest regulatory activity in relation to ubiquinolcytochrome-c-oxidoreductase is possessed by 3 - [(E) -3- (3,5-di-tert-butyl-4-hydroxy-phenyl) - 3-oxo-prop-1-enyl] -6-methoxy-chromen-4-one, the use of which contributed to the restoration of mitochondrial complex III (the activity was 85.2% higher ($p < 0.05$) in relation to the group of negative control). The calculated correlation coefficients of the activity level from changes in the quantum-chemical parameters of molecules showed that the inclusion of halogens in the target structure leads to a significant decrease in the pharmacological effect. The above facts allow us to assert that, in order to achieve mitochondrial effects in predicting new compounds that are 3-substituted chromone derivatives, it is not advisable to introduce halogens into the aromatic nucleus, be it annelated or as an acetophenone fragment.

5. MATERIALS AND METHODS

5.1. Synthesis of target compounds

The synthesis of 3-formylchromone is carried out according to a well-known scheme using Vilsmeier's reagent. According to this scheme (Figure 1), 3-formylchromone was obtained; 6-chloro-3-formylchromone, 6-fluoro-3-formylchromone, 6-iodo-3-formylchromone, 6-acetoxy-3-formylchromone, 7-acetoxy-3-formylchromone, 7-methoxy-3-formylchromone. 2- [methyl (phenyl) amino] -4-oxo-4H-1-benzopyran-3-carbaldehyde was obtained by the reaction of 2-anilino-3-formylchromone and methyl iodide. The oximes of the corresponding derivatives of 3-formylchromone were obtained by reaction with hydroxylamine (Figure 2) [19]. The following reagents were used during the synthesis: phosphorus (V) oxychloride POCl₃ (99 %, Merk), anhydrous dimethylformamide (99 %, Merk), anhydrous acetone (Pancreac), petroleum ether (Pancreac), hydroxylamine hydrochloride (99 %, Merk). The compounds were recrystallized fivefold from absolute ethanol.

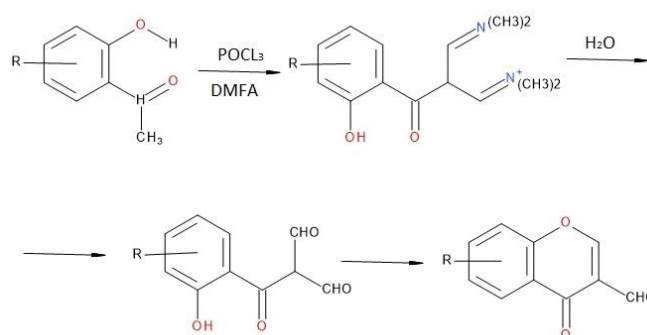


Figure 1. Scheme of the 3-formylchromone synthesis

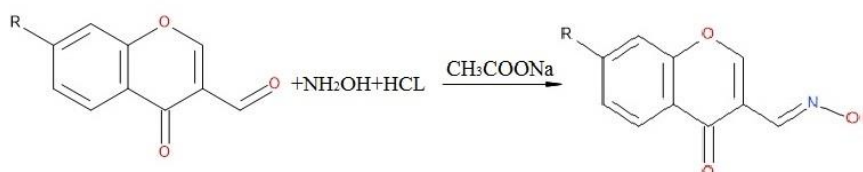


Figure 2. Scheme of the oximes of the corresponding derivatives of 3-formylchromone) synthesis

Derivatives of 1-phenyl-3-oxoprop-1-en-1-yl-1- (4H-1-benzopyran-4-one) were obtained in the reaction of 3-formylchromone and the corresponding acetophenone. 3 - [(E)-3-(3,5-di-tert-butyl-4-hydroxy-phenyl) -3-oxo-prop-1-enyl] -6-methoxy-chromen-4-one was obtained in reaction 6-methoxy-3-formylchromone and 3,5-di-tert-butyl-4-hydroxyacetophenone (Figure 3) [19]. The following reagents were used during the synthesis: acetophenones (Sigma-Aldrich), glacial acetic acid (Pancreac), concentrated sulfuric acid (Pancreac) The compounds were recrystallized fivefold from absolute ethanol.

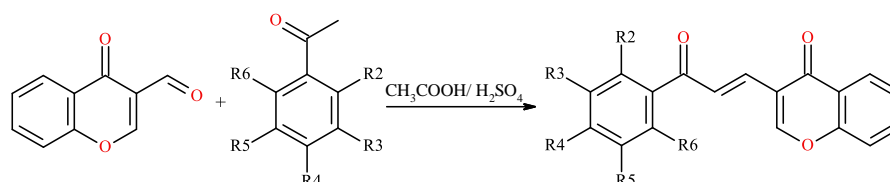


Figure 3. Scheme of the 1-phenyl-3-oxoprop-1-en-1-yl-1- (4H-1-benzopyran-4-one) synthesis

The substances obtained were identified by IR, UV and ¹H NMR spectroscopy, as well as using physicochemical methods of analysis (determination of the melting point). NMR spectroscopy (the substances were dissolved in dimethyl sulfoxide without heating) was performed using the NTEGRA SPECTRA complex

(Russia), IR (in vaseline oil) and UV (the substances were dissolved in dimethyl sulfoxide without heating, absorbance was measured against pure dimethyl sulfoxide) spectroscopy was performed using the systems of the FTIR spectrometer FSM-1201 (Russia) and the spectrophotometer SF-102 (Russia). The melting point of the analyzed samples was evaluated on an M-560 analyzer with the use of quartz glass capillaries.

The structural formulas of the test-compounds are given in Table 4.

3-formylchromone (C3A)

Yield -85%. $^1\text{H NMR}$ (400 MHz, DMSO- d_6), δ 7.43-7.55 (m, 2H, Ar); 7.73-7.79 (m, 1H, Ar); 8.29 (dd, 1H, Ar); 8.55 (s, 1H, Ar); 10.38 (s, ^1H , CHO); IR spectrum, ν C = O (CHO) 1695 cm^{-1} , ν C = O 1650 cm^{-1} , ν C = C 1620 cm^{-1} (vase oil); UV spectrum: λ_{max} 203, 223, 295 nm (ethanol); Tm 165°C. Elemental analysis data (%) for $\text{C}_{10}\text{H}_6\text{O}_3$ (174.15): Found: C, 68.5; H, 3.9; O, 27.6. Calculated: C, 69; H, 3.5; O, 27.6.

6-chloro-4-oxo-4H-1-benzopyran-3-carbaldehyde (C3ACL)

Yield -75%. $^1\text{H NMR}$ (400 MHz, DMSO- d_6), δ 7.43-7.55 (m, 2H, Ar); 7.73-7.79 (m, 1H, Ar); 8.29 (dd, 1H, Ar); 8.55 (s, 1H, Ar); 10.38 (s, ^1H , CHO); Tm 167-168°C; IR spectrum, ν C = O (CHO) 1695, ν C = O chromone 1660, ν C = C 1610 cm^{-1} (vaseline oil); UV spectrum: λ_{max} 224, 313, 346 nm (ethanol). Elemental analysis data (%) for $\text{C}_{10}\text{H}_5\text{ClO}_3$ (208.59): Found: C, 58; H, 1.9; Cl, 16.9; O, 23.2. Calculated: C, 57.6; H, 2.4; Cl, 17; O, 23.

6-fluoro-4-oxo-4H-1-benzopyran-3-carbaldehyde (C3AF)

Yield -67%. $^1\text{H NMR}$ (400 MHz, DMSO- d_6), δ 7.47-7.52 (m; 1H; Ar); 7.58-7.61 (q, 1H, Ar); 7.69-7.9 (q, 1H, Ar); 8.57 (s, 1H, Ar); 10.37 (s, ^1H , CHO); Tm 152-154°C; IR spectrum, ν C = O (CHO) 1695, C = O chromone 1655, C = C 1605 cm^{-1} (vaseline oil); UV spectrum: λ_{max} 203, 224, 302 nm (ethanol). Elemental analysis data (%) for $\text{C}_{10}\text{H}_5\text{FO}_3$ (192.14): Found: C, 62.3; H, 2.5; F, 9.8; O, 25.4. Calculated: C, 62.5; H, 2.6; F, 9.9; O, 25.

6-iodo-4-oxo-4H-1-benzopyran-3-carbaldehyde (C3AI)

Yield -81%. $^1\text{H NMR}$ (400 MHz, DMSO- d_6), δ 7.28 (s, 1H, Ar); 8.49 (s, 1H, Ar); 8.59 (d, 1H, Ar); 8.62 (s, 1H, Ar); 10.37 (s, ^1H , CHO). ($^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ = 7.60 (d, 1H, J = 8.8 Hz), 8.18 (dd, 1H, J = 2.4 and 8.8 Hz), 8.37 (d, 1H, J = 2.4 Hz), 8.95 (s, 1H), 10.10 (s, 1H).); Tm - 219°C; IR spectrum, ν C = O (CHO) 1695, ν C = O chromone 1660, ν C = C 1605 cm^{-1} (vaseline oil); UV spectrum: λ_{max} 248, 286, 325 nm (ethanol). Elemental analysis data (%) for $\text{C}_{10}\text{H}_5\text{IO}_3$ (300.04): Found: C, 39.7; H, 2; I, 42; O, 16.3. Calculated: C, 40; H, 1.7; I, 42.3; O, 16.

3-formyl-4-oxo-4H-1-benzopyran-6-yl acetate (C3A6Ac)

Yield -65%. $^1\text{H NMR}$ (400 MHz, DMSO- d_6), δ 2.39 (s, 3H, CH₃); 7.53 (dd, 1H, Ar); 7.59 (dd, 1H, Ar); 8.02 (d, 1H, Ar); 8.57 (s, 1H, Ar); 10.39 (s, ^1H , CHO); Tm 153°C; IR spectrum, ν C = O (CHO) 1698, C = O 1649, OCOCH₃ 1770 cm^{-1} (vase oil); UV spectrum: λ_{max} 203, 227, 304 nm (ethanol) Elemental analysis data (%) for $\text{C}_{12}\text{H}_8\text{O}_5$ (232.18): Found: C, 62.3; H, 3.6; O, 34.6. Calculated: C, 62.1; H, 3.5; O, 34.5.

3-formyl-4-oxo-4H-1-benzopyran-7-yl acetate (C3A7Ac)

Yield -69%. $^1\text{H NMR}$ (400 MHz, DMSO- d_6), δ 2.39 (s, 3H, CH₃); 7.27 (d, 1H, Ar); 7.40 (s, 1H, Ar); 8.34 (d, 1H, Ar); 8.55 (s, 1H, Ar); 10.40 (s, ^1H , CHO); Tm.154-155°C; IR spectrum, ν C = O (CHO) 1696, C = O 1647, OCOCH₃ 1769 cm^{-1} (vase oil); UV spectrum: λ_{max} 203, 223, 293 (ethanol). Elemental analysis data (%) for $\text{C}_{12}\text{H}_8\text{O}_5$ (232.18): Found: C, 62.3; H, 3.6; O, 34.6. Calculated: C, 62.1; H, 3.5; O, 34.5.

7-methoxy-4-oxo-4H-1-benzopyran-3-carbaldehyde (C3AOCH3)

Yield -67%. $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 3.93 (s, 3H, OCH₃); 6.93-7.55 (s, 1H, Ar); 7.07 (dd, 1H, Ar); 8.21 (d, 1H, Ar); 8.50 (s, 1H, Ar); 10.39 (s, ^1H , CHO); Tm 195°C; IR spectrum, ν C = O (CHO) 1695, C = O chromone 1655, C = C 1605 cm^{-1} (petrolatum oil); UV spectrum: λ_{max} 211, 246, 295 (ethanol). Elemental analysis data (%) for $\text{C}_{11}\text{H}_8\text{O}_4$ (204.18): Found: C, 64.3; H, 4.1; O, 31.5. Calculated: C, 64.7; H, 3.9; O, 31.3.

2- [methyl (phenyl) amino] -4-oxo-4H-1-benzopyran-3-carbaldehyde (C3ACH3Phen)

Yield –85% ¹H NMR (400 MHz), δ, ppm in DMSO-d₆: 4.07 (s, 3H, CH₃); 7.41-7.46 (m, 5H, Ar); 7.61-7.67 (m, 3H, Ar); 7.95 (d, 1H, Ar); 10.14 (s, 1H, CHO). T_m 168-169 °C; UV spectrum λ_{max} 325, 286, 248 nm (CH₃OH); IR spectrum (KBr) 1670, 1631, 1621, 1518 cm⁻¹. C = O (CHO) 1664, C = C 1623

3 - [(E) - (hydroxyimino) methyl] -4H-1-benzopyran-4-one (C3ANOH)

Yield –42%. ¹H NMR (400 MHz, DMSO-d₆) δ 11.43 (s, 1H, OH), 8.69 (s, 1H, CH = N), 8.14 - 8.06 (m, 2H, ArH), 7.85 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H, ArH), 7.70 (d, J = 8.5 Hz, 1H, ArH), 7.58 - 7.50 (m, 1H, ArH); T_m = 116-117 °C; IR spectrum, ν C = O (CHO) 1660, C = N 945, C = C 1618 cm⁻¹ (liquid petroleum); UV spectrum: λ_{max} 216, 250, 307 (ethanol). Elemental analysis data (%) for C₁₀H₇NO₃ (189.16): Found: C, 63.3; H, 3.9; N, 7.3; O, 25.5 Calculated: C, 63.5; H, 3.7; N, 7.4; O, 25.4.

(3E) -6-fluoro-4-oxo-chromene-3-carbaldehyde oxime (C3AFNOH)

Yield –92%. ¹H NMR spectrum (400 MHz), δ, ppm in DMSO-d₆: 7.59-7.82 (m; 3H; Ar); 8.05 (s, 1H, Ar); 8.69 (d, 1H, CH); 11.45 (s, 1H, OH); T_m = 220-222 °C; IR spectrum, ν C = O (CHO) 1652, C = N 954, C = C 1623 cm⁻¹ (vase oil); UV spectrum: λ_{max} 202, 253, 310 (ethanol). Elemental analysis data (%) for C₁₀H₆FNO₃ (207.16): Found: C, 58.3; H, 2.6; F 9.0; N, 6.7; O, 23.5. Calculated: C, 58; H, 2.9; F 9.2; N, 6.8; O, 23.2.

(3E) -6-chloro-4-oxo-chromene-3-carbaldehyde oxime (C3ACLNOH)

Yield –80%. ¹H NMR spectrum (400 MHz), δ, ppm in DMSO-d₆: 7.74-7.87 (m; 3H; Ar); 8.02-8.06 (q, 1H, Ar); 8.67 (d, 1H, CH); 11.55 (d, 1H, OH); T_m = 199-200 °C; IR spectrum, ν C = O (CHO) 1656, C = N 954, C = C 1623 cm⁻¹ (vase oil); UV spectrum: λ_{max} 203, 253, 316 (ethanol). Elemental data analysis (%) for C₁₀H₆ClNO₃ (223.61): Found: C, 53.3; H, 2.6; Cl 16.0; N, 6.5; O, 21.7. Calculated: C, 53.7; H, 2.7; Cl 15.9; N, 6.3; O, 21.5.

3 - [(1E) -3-oxo-3-phenylprop-1-en-1-yl] -4H-1-benzopyran-4-one (C3AACP1)

Yield – 21%. ¹H NMR (400 MHz, Chloroform-d) δ 8.70 (d, J = 15.4 Hz, 1H), 8.32 (d, J = 7.9 Hz, 1H), 8.22 (s, 1H), 8.12 (d, J = 7.9 Hz, 2H), 7.73 (t, J = 7.8 Hz, 1H), 7.59 (t, J = 6.9 Hz, 1H), 7.50 (q, J = 7.5 Hz, 5H); T_m = 171-172°C; IR spectrum, ν C = O (propenone) 1661, C = O (CHO chromone) 1612, C = C (propenone) 1589, C = C (propenone) 1003 cm⁻¹ (liquid petrolatum); UV spectrum: λ_{max} 309, 280, 229 (ethanol). Elemental analysis data (%) for C₁₈H₁₂O₃ (276.2): Found: C, 78.1; H, 4.3; O, 17.6. Calculated: C, 78; 2 H, 4.4; O, 17.4.

3 - [(1E) -3- (3,4-dimethylphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one (C3AACP2)

Yield – 33%. ¹H NMR (400 MHz, Chloroform-d) δ 8.68 (d, J = 15.3 Hz, 1H, ArH), 8.32 (dd, J = 8.1, 1.7 Hz, 1H, ArH), 8.21 (s, 1H, ArH), 7.88 (d, J = 10.9 Hz, 2H, ArH), 7.78 - 7.67 (m, 1H, CH = CH), 7.50 (dd, J = 14.7, 7.5 Hz, 3H, ArH), 7.28 (d, J = 5.2 Hz, 1H, CH = CH), 2.35 (d, J = 5.2 Hz, 6H, CH₃); T_m = 181-182°C; IR spectrum, ν C = O (propenone) 1665, C = O (CHO chromone) 1603, C = C (propenone) 1577, C = C (propenone) 999 cm⁻¹ (liquid petrolatum); UV spectrum: λ_{max} 230, 309 (ethanol). Elemental analysis data (%) for C₂₀H₁₆O₃ (304.3): Found: C, 78.8; H, 5.4; O, 15.8. Calculated: C, 78; 9H, 5.3; O, 15.8.

3 - [(1E) -3- (2-hydroxy-5-methylphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one (C3AACP3)

Yield – 60%. ¹H NMR (400 MHz, Chloroform-d) δ 12.70 (s, 1H, OH), 8.82 (d, J = 15.2 Hz, 1H, ArH), 8.34 (dd, J = 8.0, 1.7 Hz, 1H, ArH), 8.24 (s, 1H, ArH), 7.81 (d, J = 2.2 Hz, 1H, ArH), 7.79 - 7.71 (m, 1H, C = H), 7.58 - 7.48 (m, 3H, ArH), 7.32 (dd, J = 8.4, 2.2 Hz, 1H, ArH), 6.93 (d, J = 8.4 Hz, 1H, C = H), 2.37 (s, 3H, CH₃); T_m = 205°C; IR spectrum, ν C = O (propenone) 1665, C = O (CHO chromone) 1644, C = C (propenone) 1573, C = C (propenone) 998 cm⁻¹ (liquid petrolatum); UV spectrum: λ_{max} 312, 229 (ethanol). Elemental analysis data (%) for C₁₉H₁₄O₄ (306.31): Found: C, 74.3; H, 4.8; O, 20.9. Calculated: C, 74; 5 H, 4.6; O, 20.9.

3 - [(1E) -3- (5-fluoro-2-hydroxyphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one (C3AACP4)

Yield – 47%. ¹H NMR (400 MHz, Chloroform-d) δ 12.58 (s, 1H, OH), 8.76 (d, J = 15.1 Hz, 1H, ArH), 8.38 - 8.31 (m, 1H, ArH), 8.27 (s, 1H, CH =), 7.81 - 7.69 (m, 2H, ArH), 7.62 - 7.49 (m, 4H, ArH), 7.00 (dd, J = 9.2, 4.8 Hz, 1H, C = H); T_m = 208°C; IR spectrum, ν C = O (propenone) 1661, C = O (CHO chromone) 1612, C = C (propenone) 1577, C = C 1623 cm⁻¹ (liquid petrolatum); UV spectrum: λ_{max} 315, 234 (ethanol). Elemental analysis data (%) for C₁₈H₁₁FO₄ (310.3): Found: C, 69.6; H, 3.5; F 6.2; O, 20.7. Calculated: C, 69; 7 H, 3.6; F 6.1; O, 20.6.

3 - [(1E) -3- (2-hydroxy-3-iodo-5-methylphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one (C3AACP5)

Yield – 60%. ¹H NMR (400 MHz, Chloroform-d) δ 13.63 (s, 1H, OH), 8.82 (d, J = 15.1 Hz, 1H, ArH), 8.31 (dd, J = 8.0, 1.7 Hz, 1H, ArH), 8.23 (s, 1H, CH = CH), 7.81 (s, 2H, ArH), 7.78 - 7.72 (m, 1H, CH = CH), 7.60 - 7.47 (m, 3H, ArH), 2.34 (s, 3H, CH₃); T_m = 223°C; IR spectrum, ν C = O (propenone) 1650, C = O (CHO chromone) 1628, C = C (propenone) 1554, C = C (propenone) 979 cm⁻¹ (liquid petrolatum); UV spectrum: λ_{max} 322, 238 (ethanol). Elemental analysis data (%) for C₁₉H₁₃IO₄ (432.2): Found: C, 52.7; H, 3.1; I 29.3; O, 14.9. Calculated: C, 52; 8 H, 3; I 29.4; O, 14.8.

3 - [(1E) -3- (2-hydroxy-4-methoxyphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one (C3AACP6)

Yield- 60%. ¹H NMR (400 MHz, Chloroform-d) δ 12.48 (s, 1H, OH), 8.79 (d, J = 15.1 Hz, 1H, ArH), 8.34 (dd, J = 8.0, 1.7 Hz, 1H, CH = CH), 8.25 (s, 1H, ArH), 7.76 (ddd, J = 8.7, 7.0, 1.8 Hz, 1H, ArH), 7.60 - 7.47 (m, 4H, ArH), 7.16 (dd, J = 9.0, 3.0 Hz, 1H, CH = CH), 6.98 (d, J = 9.1 Hz, 1H, ArH), 3.89 (s, 3H, CH₃); T_m = 201°C; IR spectrum, ν C = O (propenone) 1663, C = O (CHO chromone) 1644, C = C (propenone) 1592, C = C (propenone) 980 cm⁻¹ (liquid petrolatum); UV spectrum : λ_{max} 395, 313, 231 (ethanol) Elemental analysis data (%) for C₁₉H₁₄O₅ (322.3): Found: C, 70.7; H, 4.5; O, 24.9. Calculated: C, 70; 8 H, 4.4; O, 24.8.

3 - [(E) -3- (3,5-di-tert-butyl-4-hydroxy-phenyl) -3-oxo-prop-1-enyl] -6-methoxy-chromen-4-one (C3AACP7)

Yield – 22% ¹H NMR spectrum (400 MHz), δ, ppm in DMSO-d₆: 1.53 (s, 18H, 6CH₃); 3.95- (s, 3H, OCH₃); 5.79 (s, 1H, C = H); 7.28-7.32 (d, J = 15.3, 1H, C = H); 7.46-7.48 (d, J = 3.1, 2H, ArH); 7.68-7.69 (d, J = 3.0, 1H, chromone); 8.02 (s, 2H, chromone); 8.21 (s, 1H, chromone); 8.64 (d, 1H, OH). T_m. 238°C; IR spectrum, ν C = O (propenone) 1661, C = O (CHO chromone) 1636, C = C (propenone) 1593, C = C (propenone) 998 cm⁻¹ (petrolatum. oil); UV spectrum: λ_{max} 243, 325 (ethanol) Elemental analysis data (%) for C₂₇H₃₀O₅ (434.5): Found: C, 74.5; H, 7.1; O, 18.4. Calculated: C, 74; 6 H, 7; O, 18.4.

Table 4. Test-compounds formulas.

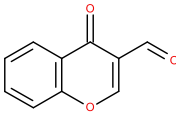
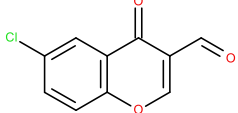
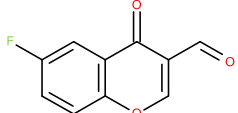
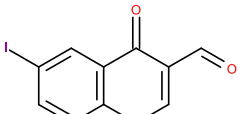
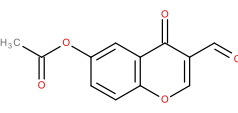
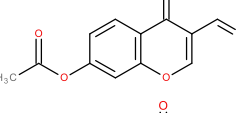
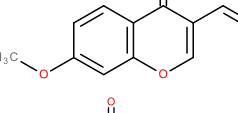
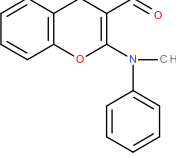
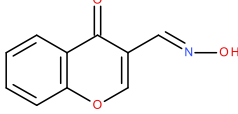
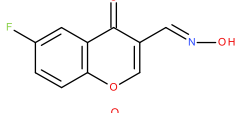
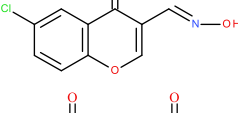
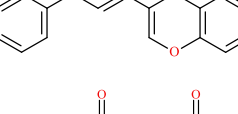
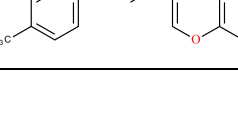
Formula	IUPAC	Laboratory code
	3-formylchromone	C3A
	6-chloro-4-oxo-4H-1-benzopyran-3-carbaldehyde	C3ACL
	6-fluoro-4-oxo-4H-1-benzopyran-3-carbaldehyde	C3AF
	6-iodo-4-oxo-4H-1-benzopyran-3-carbaldehyde	C3AI
	3-formyl-4-oxo-4H-1-benzopyran-6-yl acetate	C3A6Ac
	3-formyl-4-oxo-4H-1-benzopyran-7-yl acetate	C3A7Ac
	3-methoxy-4-oxo-4H-1-benzopyran-3-carbaldehyde	C3AOCH3
	2-[methyl (phenyl) amino]-4-oxo-4H-1-benzopyran-3-carbaldehyde	C3ACH3Ph en
	3-[(E)-(hydroxyimino)methyl]-4H-1-benzopyran-4-one	C3ANOH
	(3E)-6-fluoro-4-oxo-chromene-3-carbaldehyde oxime	C3AFNOH
	(3E)-6-chloro-4-oxo-chromene-3-carbaldehyde oxime	C3ACLNO H
	3-[(1E)-3-oxo-3-phenylprop-1-en-1-yl]-4H-1-benzopyran-4-one	C3AACP1
	3-[(1E)-3-(3,4-dimethylphenyl)-3-oxoprop-1-en-1-yl]-4H-1-benzopyran-4-one	C3AACP2

Table 4. Test-compounds formulas. (continued)

Formula	IUPAC	Laboratory code
	3 - [(1E) -3- (2-hydroxy-5-methylphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one	C3AACP3
	3 - [(1E) -3- (5-fluoro-2-hydroxyphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one	C3AACP4
	3 - [(1E) -3- (2-hydroxy-3-iodo-5-methylphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one	C3AACP5
	3 - [(1E) -3- (2-hydroxy-4-methoxyphenyl) -3-oxoprop-1-en-1-yl] -4H-1-benzopyran-4-one	C3AACP6
	3 - [(E) -3- (3,5-di-tert-butyl-4-hydroxy-phenyl) -3-oxo-prop-1-enyl] -6-methoxy-chromen-4-one	C3AACP7

5.2. Laboratory animals

Evaluation of pharmacological activity was performed on male Wistar rats weighing 200-220 grams, 3 months (n = 10 in each experimental group). The animals were obtained from the Rappolovo laboratory animal nursery (Russia, Leningrad region) and during the experiment were kept under controlled conditions in the laboratory of living systems of the Pyatigorsk Medical and Pharmaceutical Institute. The maintenance and all manipulations carried out with the animals complied with the recommendations of Directive 2010/63 / EU of the European Parliament and of the council on the protection of animals used for scientific purposes, September 22, 2010 and ARRIVE guidelines [20]. The research concept was approved by the local ethics committee (meeting minutes No. 10 of 12/12/2020).

5.3. Cerebral ischemia model

Cerebral ischemia was modeled by the method of irreversible occlusion of the middle cerebral artery. The course of the operation: in anesthetized animals (chloral hydrate 350 mg / kg, intraperitoneally), on the depilated area below and to the right of the eye, the skin was dissected and the muscles were moved apart. Then the process of the zygomatic bone was removed and the skull was exposed. Next, a burr was used to make a trepanation hole above the intersection of the middle cerebral artery and the olfactory tract, the dura mater was removed, and the artery was electrocoagulated, followed by cutting to avoid vessel recanalization, and the wound was sutured in layers. The seam was treated with an antiseptic solution. Before awakening, the animals were left under a warming lamp [21].

CoQ10 (Hunan Warrant Pharm, China) at a dose of 200 mg / kg (per os) was used as a reference drug [22]. The compounds under study were administered at a dose of 40 mg / kg orally for 3 days after modeling brain ischemia (the reference was administered in the same way) [9]. The compounds under study were administered to intact animals for 10 days [9].

5.4. Biomaterial preparation

When studying the activity of mitochondrial complex III, the brain of animals was used as a biomaterial, which was homogenized in the isolation medium (1 mmol EGTA + 215 mmol mannitol + 75 mmol sucrose + 0.1% BSA solution + 20 mmol HEPES with pH 7.2). The resulting homogenate was centrifuged at 1100 g for 2 minutes. Secondary supernatant in the amount of 700 µl was transferred into Eppendorf tubes, mixed with 75

μ l of 10% Percol and centrifuged at 18000g for 10 minutes. The pellet was resuspended in 1 ml of isolation medium and centrifuged for 5 minutes at 10,000 g [23].

5.5. Methods for assessing the activity of the mitochondrial complex III

The activity of complex III was assessed by a spectrophotometric method based on an increase in the optical density (550 nm) of a solution containing succinate, cytochrome C, rotenone, and an analyzed sample. The activity of the complex was expressed in terms of the protein concentration in the analyzed sample. The protein content was estimated according to the Bradford method [24].

5.6. Methods for calculating quantum chemical parameters

Calculation of quantum chemical parameters: Mulliken charges (a.u.) on atoms forming a specific structure, bond numbers (N_{μ}), theoretical valence (V_{μ}), unsaturation index (IUA), electron density, free valence index (F_{μ}), were calculated by the semiempirical PM7 method (WinMopac 2016) using an IntelXeonES-1620 3.5 Hz processor, 20 GB of RAM.

5.7. Statistical Methods

Statistical processing of the research results was carried out using the applied software packages MS Excel 2013 and STATISTICA 6.0 (StatSoft, USA) for Windows. Data were expressed as M (mean) \pm SEM (standard error of the mean). Statistically significant differences between groups were assessed by one-way analysis of variance (ANOVA) with post-Newman-Keuls post-processing at a significance level of $p < 0.05$. Correlation analysis was performed using Spearman's test.

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Conflict of interest statement: Authors declare no conflict of interest

Ethical approval: The study was approved by the local ethics committee (protocol No. 10 of 12/12/2020).

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